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I. SYNTHESIS OF DERIVATIVES OF 2,6-DIAMINO-2,6-DIDEOXY-D-MANNOSE AND 2-AMINO-3,6-ANHYDRO-2-DEOXY-D-MANNOSE. II. AMINO DERIVATIVES OF STARCHES. ATTEMPTED SYNTHESIS OF 2-AMINO-3,6-ANHYDRO-2-DEOXY-AMYLOSE AND DEGRADATIVE EXPERIMENTS ON N-ACETYLATED AMYLOSE.

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DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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The Ohio State University
1966

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INTRODUCTION

Amino sugars or glycosamines are carbohydrates containing amino groups in place of alcoholic hydroxyl groups, and differ from glycosylamines (N-glycosides), in which the amino group takes the place of a hemiacetal hydroxyl. A characteristic difference between amino sugars and glucosylamines is generally the stability of the carbon-nitrogen bond in the former and the lability of this bond in the latter (1,2).


Compounds genetically related to the amino sugars are their reduction products — alditols containing amino groups — the amino sugar alditols. Amino sugar alditols having an amino group on a primary carbon atom are usually called glycamines. Finally, compounds similar to the amino sugars and amino sugar alditols are the amino derivatives of cyclitols, the polyhydroxycyclohexanes, or the inosamines (3,4).

For a long time only two amino sugars — both of natural origin — were known: 2-amino-2-deoxy-D-glucose (5) and 2-amino-2-deoxy-D-galactose (6), whose configuration was established (7) in 1937-1939

(5) G. Ledderhose, Ber., 52, 1200 (1876).

(6) P. A. Levene and F. B. LaForge, J. Biol. Chem., 18, 123 (1914).


and (8) in 1945. Individual, synthetic amino sugars and amino sugar alditols were prepared by Fischer and co-workers (9,10), Freudenberg,

(9) E. Fischer and H. Leuchs, Ber., 36, 24 (1903).

(10) E. Fischer and K. Zach, Ber., 44, 132 (1911).

Helferich, Ohle, and others. Particular interest in the amino sugars was aroused by the discovery of 2-deoxy-L-2-methylamino-glucose in the products arising from the extensive degradation of the antibiotic streptomycin (11); later, other amino sugars were found in a number

of antibiotics. Individual inosamines were also found as components of antibiotics (3). At the same time increasing attention was drawn to the discovery of amino sugars in biologically important polysaccharides and proteins.

The importance of amino sugars at the present time is due to the fact that they are present in important macromolecular substances which are found in living organisms and in a number of cases exhibit biological activity. They are present in bacterial organisms, shells of crustacea, insects, fungi, and soil polysaccharides. Amino sugars are components of a number of antibiotics and of the oligosaccharides of human milk. Work is being carried out on the use of amino sugars and amino sugar alditols as drugs, surface-active agents and starting materials for the preparation of polymers.

A number of reviews (12-21) and reference tables (22) devoted


partly or entirely to amino sugars have been published. A few reviews on the amino polysaccharides have also appeared (17,18,23-27).


Amino sugars are obtained from the degradation of a number of complex natural products present in animal and vegetable organisms, micro organisms, soil substances, and antibiotics. In natural substances the primary amino group of the amino sugars is usually acylated; most frequently with an acetic acid residue but also with the residue of sulfuric acid and other acids.

In nature, together with 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose, which are the most commonly encountered amino sugars, the other amino sugars are also in most cases amino aldohexoses. Only a few representatives of other types of amino sugars are encountered. Deoxygenated amino sugars with methylene and methyl groups are fairly widespread, and N-alkylated (methylated) amino sugars and
diamino sugars are also encountered. In addition to amino sugars in
the true sense, natural substances also contain the amino sugar acids:
2-amino-2-deoxy-D-galacturonic acid, muramic acid (I), and sialic
acids - the $N$-acyl derivatives of neuraminic acid (II). The latter
are genetically related to 2-amino-2-deoxy-D-mannose which are formed
when these acids are degraded.

In living organisms, amino sugars are found chiefly in the so-
called "mucoid" substances. These include 2-amino-2-deoxy-D-glucose,
2-amino-2-deoxy-D-galactose, and sialic acids together with other sugars,
hexuronic acids, amino acids, and lipids. "Mucoid" substances are more
subdivided into what are more correctly termed, glycopolysaccharides,
glycoproteins, and glycolipids, depending on the quantity of the corre-
sponding components present.

The amino sugars found in bacteria include, in addition to 2-amino-
2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, muramic acid and sialic
acidss, other less common amino sugars: 2-amino-2,6-dideoxy-D-
galactose and -L-galactose, and the like. Here the amino sugars are
components of polysaccharides (26,27).

An even greater variety of amino sugars is found in antibiotics,
chiefly macrolides, streptomycins, and streptomycin-like antibiotics
(28). Antibiotics containing amino sugars generally have the chemical
structure of glycosides of amino sugars; the antibiotic puromycin is a nucleoside of 3-amino-3-deoxy-D-ribose.

Work carried out in recent years has reduced considerably the list of amino sugars of unknown configuration and has revealed the nature of many amino sugars present in antibiotics. Thus it has been shown that the N-dimethylaminohexose, mycaminose, found in magnamycin (carbomycin) and other antibiotics and previously assigned the configuration of D-altrose, is in fact 3,6-dideoxy-3-dimethylamino-D-glucose (29,30). Mycosamine (the amino hexose of the antibiotics nystatin, pimaricin, and others) is 3-amino-3,6-dideoxy-D-mannose (31,32).

The structure of two other amino sugars present in antibiotics - amosamine and rhodosamine - has recently been established. The former, a 4-aminohexose, is 4,6-dideoxy-4-dimethylamino-D-glucose (33).
structure of glycosides of amino sugars; the antibiotic puromycin is a nucleoside of 3-amino-3-deoxy-D-ribose.

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The structure of two other amino sugars present in antibiotics — amosamine and rhodosamine — has recently been established. The former, a 4-aminohexose, is 4,6-dideoxy-4-dimethylamino-D-glucose (33).
and the latter is 2,3,6-trideoxy-3-dimethylamino-L-lyxo-hexose (34).

It has been established by degradation, study of the nuclear magnetic resonance spectra, and synthesis that the extremely widespread amino sugar desosamine is 3,4,6-trideoxy-3-dimethylamino-D-xylo-hexose (35-38).

Among the substances related to amino sugars and found in natural products, not including riboflavin, are the amino cyclitols: streptamine (III) (in streptomycin, and related antibiotics), 2-deoxystreptamine (IV) (in kanamycins, neomycins, and paromomycin), neoinosamine-2 (V) (in hygromycin) (3), 2-deoxy-N-methylstreptamine (VI) (in hygromycin B) (39) and tetrahydroxy-1,3-dimethylaminocyclohexane (in the antibiotic actinospectacin (40).


DEVELOPMENT OF THE PROBLEM

The introduction of substituents such as amino, sulfonyl, halogen, or other groups, into the starch molecule would alter its physical and chemical properties, and would offer possibilities of wider utilization. It is of interest to perform chemical modifications on the amylose molecule with a view to the replacement of secondary hydroxyl by amino groups while maintaining polymeric structure. Such a cationic polymer might be expected to possess physical and chemical properties which could offer possibilities for increased utilization of starch.

A polymer modified by amination at C-2 might possess the high stability toward hydrolysis exhibited by chitosan; the acetamido analog would be analogous to chitin, a polysaccharide whose high degree of intermolecular hydrogen bonding (41) affords great physical stability.


An amino group may be introduced into a sugar molecule by treatment of a suitably substituted sugar with ammonia (42-44) or an amine

(43) L. F. Wiggins, ibid., 522 (1944).
(44) K. Freudenberg, O. Burkhart, and E. Braun, Ber., 52, 714 (1926).
Sulfonate groups (42-45) and halogen groups (3) may be replaced by amino groups in this manner, often through intermediate epoxide derivatives. Sugar epoxides are thus also useful intermediates in amination reactions, and are readily obtainable from suitable sulfonate esters or halogeno derivatives (46,47). Amination in predetermined locations requires intermediates substituted in a known manner. Sulfonation of sugars with, for instance, p-toluene-sulfonyl chloride or methanesulfonyl chloride, will, with an excess of reagent, cause esterification of all hydroxyl groups, while selective sulfonation of primary hydroxyl groups may be achieved with less reagent. With suitable blocking groups sulfonation may be restricted to selected secondary hydroxyl groups. The examples on the following page are illustrative.
When the sulfonate ester group is "isolated" (48) (that is, when no adjacent hydroxyl or alkali-labile substituted hydroxyl group is present), as in IV above, it will undergo displacement with ammonia under vigorous conditions (44) with Walden inversion (49,50) to give the amino sugar derivatives, for example V.


Hydrazinolysis (51,52), followed by reduction (50,53-55) achieves

(51) K. Freudenberg and F. Brauns, Ber., 55, 3233 (1922).
(52) K. Freudenberg and A. Doser, ibid., 56, 1243 (1923).

the same net result under milder conditions as in the following example (51).
A side reaction (51) which occurs under these conditions causes the formation of an olefin by a trans-elimination mechanism.

\[
\begin{align*}
\text{IV} & \quad \rightarrow \\
& \\
& + \text{TsOH} \\
\text{VII}
\end{align*}
\]

This reaction offers a potentially useful route to an intermediate which would undoubtedly be reactive in addition type reactions.

The displacement of halogen atom by amines (10) has received little attention in the carbohydrates, but by analogy with examples in the cyclitol (3) series the reaction should take place under similar conditions to those used in the displacement of the sulfonate esters. The recent work of Jones and co-workers (56, 57) has shown


that treatment of methyl glycosides with sulfuryl chloride can be used to convert secondary hydroxyl groups to chloro-deoxy substituents, and simultaneously esterify other hydroxyl groups to form sulfate half esters, without cleavage of the glycosidic linkage.

Treatment of sulfonate esters or chloro-deoxy derivatives of sugars with base under mild conditions will, if suitably placed
hydroxyl groups are available, cause the formation of anhydro derivatives (46-48, 56-58).


Thus for example, methyl 6-bromo-6-deoxy-α-D-glucopyranoside (VIII) (59) gives the 3,6-anhydro derivative (IX). This reaction has been used (60) for blocking the 3 and 6 positions in the starch molecule, as in the following example.


Such anhydro derivatives would not normally undergo further reaction leading to ring opening, and would be regarded as fixed blocking groups. If an adjacent trans-related hydroxyl group is involved in anhydro formation, the reactive epoxide derivative is formed, as in the following examples (46, 47, 56, 57, 61, 62):


The direction of the epoxide formation can be controlled by the use of suitably placed alkali-stable substituents (12-14,18).

The epoxide derivatives are reactive and readily undergo ammonolysis

to give isomeric vicinal amino alcohols (12,14,18,46,47,58). The direction of epoxide ring opening in sugar rings (except epoxides involving the terminal nonreducing carbon) is dependent on stereochemical considerations (7,12-14,18,47,63,64).


Thus:

Aminolysis of sugar sulfonate esters (and presumably, halogen derivatives) which have hydroxyl groups suitably placed for epoxide formation, yield amino sugars directly, by simultaneous epoxide formation by the basic environment and scission by the amine (12, 14, 18, 46, 47).

Like ammonia or hydrazine, an azide can be used for direct displacement of a sulfonloxy group in a sugar molecule to produce the corresponding azido compound which, on hydrogenation would produce the amino sugar (65). Azide ion is a more powerful nucleophile than ammonia. Replacement of the primary sulfonloxy group by azide ion occurs readily in acetone-water (65-66) or dimethyl sulfoxide (67).

Secondary sulfonyloxy groups can be displaced with inversion by azide ion in N\_N-dimethylformamide (33,68-73). Use of the azide ion permits a cleaner reaction, without formation of disubstituted (74) or unsaturated derivatives (52,74) as in the case of hydrazinolysis, but Wolf from and co-workers (68) have demonstrated that under certain steric and electrostatic conditions the displacement reaction proceeds smoothly when hydrazine is used as the nucleophile but essentially fails when the azide ion is employed. The azide ion can also be used to open the epoxide ring to form (75,76) two azido sugars with one of them preponderant.
Recently an aminated amylose has been reported by Wolfrom and co-workers (77). Amylose was treated with a total of 2.2 molar equivalents of p-toluenesulfonyl chloride in pyridine to yield a p-toluenesulfonate ester derivative with a degree of substitution of 1.7. Esterification of amylose with p-toluencesulfonyl chloride has been shown (60) to take place selectively and readily at the C-6 position of the D-glucose units. Further reaction would presumably occur selectively, though probably not exclusively, at the C-2 hydroxyl group (78-80).

The 2(3),6-bis-O-(p-tolylsulfonyl)amylose was then refluxed with hydrazine and the resulting hydrazine derivative was reduced by Raney nickel to yield an aminated derivative with a degree of substitution of 1.4.
In the amination by a nitrogen nucleophile of a 2,6-bis-O-(p-
tolylsulfonyl)-α-D-glucopyranose unit in the (1→4)-linked amylose
chain, at least three reasonable possible mechanistic pathways may
be postulated, as shown in Figure 1. The products are shown as con-
verted to the free amino derivatives. If direct displacement of both
p-tolylsulfonyloxy groups takes place (pathway a), inversion will
occur at C-2 and a 2,6-diamino-2,6-dideoxy-D-mannose unit will be
formed. If the reagent functions as a strong base, the initial reac-
tion may involve intramolecular attack by the anion of the C-3
hydroxyl group to displace the 6-p-tolylsulfonyloxy group (pathway c)
and give a 3,6-anhydro-2-(p-tolylsulfonyl)-D-glucose unit. Further
displacement, intermolecularly, would give a 2-amino-3,6-anhydro-2-
deoxy-D-mannose unit. Alternatively (pathway b), amination may take
place by displacement at the unhindered, primary 6-position, and the
basicity of the reagent may then facilitate attack of the C-3 oxygen
on C-2 to displace the p-tolylsulfonyloxy group and give a 2,3-epoxide
with the D-manno-configuration. The latter would predictably (81-83)

(81) E. Fischer, M. Bergmann and H. Schotte, Ber., 53, 509 (1920).
Soc., 151 (1934).

suffer rapid attack at C-3 by nitrogen, and a 3,6-diamino-3,6-dideoxy-
D-altrose unit would be formed.

In this laboratory, studies have been conducted on simple sugar
derivatives to provide model systems for the reaction leading to
Figure 1.
aminated amylose, and to provide reference compounds for comparison with possible fragmentation products obtained from aminated amylose in structural studies by degradative methods.

It has been shown (84, 85) that hydrazinolysis followed by reduction of methyl 2,6-bis-0-(methylsulfonyl)-α-D-glucopyranoside (86)


(or its bis-0-(p-tolylsulfonyl) analog (87) gives methyl 3,6-diamino-


3,6-dideoxy-α-D-altropyranoside in high yield. This indicates that pathway b is probably the favored route leading to diamino sugar units in aminated amylose prepared by hydrazinolysis (77) whereas pathway c is probably not a major route in this reaction, since it has been observed (88) that methyl 3,6-anhydro-2-0-(p-tolylsulfonyl)-α-D-glucopyranoside and methyl 3,5,3',6'-dianhydro-2,2',4'-tris-0-(p-tolylsulfonyl)-β-D-maltoside are extremely resistant to hydrazinolysis.

Recently, Wolfrom and Kato (89) have made an aminated amylose


as follows: Amylose was completely blocked at position C-6 by tritylation and the 6-\(\text{O-}(\text{triphylmethyle})\text{amylose (60) was treated with four molar equivalents of p-toluenesulfonyl chloride in pyridine to yield a p-toluenesulfonate with a degree of substitution of 0.7. p-Toluene-

sulfonation of 6-\(\text{O-}(\text{triphylmethyle})\text{amylose would presumably occur selectively, although probably not exclusively, at the C-2 hydroxyl group (78-80). The so prepared, 2(3)-\(\text{O-}(p\text{-tolylsulfonyl})-6-\(\text{O-}(\text{triphylmethyle})\text{amylose was then treated with sodium azide in 95% }N,N\text{-dimethylformamide at 125° in the presence of urea (68). An azido derivative with a degree of substitution of 0.45 was obtained. This azido derivative of 6-\(\text{O-}(\text{triphylmethyle})\text{amylose was reduced to the corresponding amino derivative by lithium aluminum hydride in tetra-

hydrofuran according to the procedure of Whistler and Medcalf (90).}


for 6-amino-6-deoxyamylose. The resultant aminated amylose having a degree of substitution of 0.45 was obtained by detritylation with acidic methanol (90).

The aminated amylose on acid hydrolysis and paper chromatography revealed two ninhydrin positive zones of \(R_f\) 0.25 and 0.36. The faster moving zone was five times stronger in intensity than the slower moving one. The two zones were separated by preparative paper
chromatography. The faster moving zone crystallized and was identified as 3-amino-1,6-anhydro-3-deoxy-D-altrose hydrochloride (82,91).


As it is known that D-altrose readily undergoes 1,6-anhydro formation under acidic conditions (92,93), the 3-amino-1,6-anhydro-3-deoxy-D-


(93) Ibid., 62, 961 (1940).

altrose hydrochloride obtained was considered to have been formed from 3-amino-3-deoxy-D-altrose hydrochloride during the acid treatment.

The slower moving zone was syrupy. It gave a positive Morgan-Elson reaction. It is known that this color reaction is given by a 2-amino-2-deoxyhexose and not by a 3-amino-3-deoxyhexose (18). Accordingly, the syrup from the slower migrating band was considered to contain some 2-amino-2-deoxyhexose, probably 2-amino-2-deoxy-D-mannose hydrochloride, together with what may have been 3-amino-3-deoxy-D-altrose hydrochloride which had not undergone 1,6-anhydro formation.

There are two possible reaction paths (Figure 2) for the replacement by azide ion of the 2-p-tolylsulfonyloxy group of 2-O-(p-tolylsulfonyl)-6-O-(triphenylmethyl)amylose (XXVI): one would be normal nucleophilic replacement in which inversion would occur to form the 2-amino-2-deoxy-D-manno configuration (XXVII); the other would be
"Figure 2."
2,3-epoxy formation with subsequent ring opening by azide ion to form the 3-amino-3-deoxy-D-altro configuration (XXIX). Epoxy ring opening to form the 2-amino-2-deoxy-D-gluco configuration (XXX) is known to occur to only a limited extent, if at all (84,85).
DIAMINO SUGARS

Importance

Only two decades have elapsed since antibiotic substances were first introduced as exceedingly useful chemotherapeutic agents. Prior to the discovery of these antibiotic substances, a limited number of amino sugars were known as components of biological tissues, especially of structural elements (see Introduction). So far about fifty different antibiotic substances have been recognized to contain various mono- and diamino sugars and cyclitols (28,94). This has led to a rapid growth of interest in diamino sugars in recent years and an increasing number of diamino sugars have been synthesized both for the purpose of structural proof and for their great antibiotic activity.

(1) Neomycins.—Neomycin was first obtained by Waksman and Lechevalier from the culture fluid of Streptomyces fradiae (95). Later,

(95) S. A. Waksman and H. A. Lechevalier, Science, 109, 305 (1949).

it was shown to consist of the closely related antibiotics neomycins B and C. These are isomeric compounds and differ only in the stereochemistry of one of their components (96,97). Both substances consist
of three C₆ units and one C₅ unit linked glycosidically. A fragment composed of a diaminohexose and 2-deoxystreptamine, known as neamine, is common to neomycins B and C. The difference between these two substances resides in the second portion, known as neobiosamine B or C, which consists of D-ribose linked to a diaminohexose. The latter (98,99), which is different in the two neomycins, is known as neosamine B or C. The structure of neosamine C has been confirmed by comparison of the diamino hexose with synthetic 2,6-diamino-2,6-dideoxy-D-glucose (100-102), while neosamine B was shown to be 2,6-diamino-2,6-dideoxy-
gave the structure for neomycins B and C shown in I.

(2) Paromomycin.—Paromomycin, produced by a Streptomyces sp., resembles neomycin in its structural pattern. It contains D-glucosamine, 2-deoxystreptamine, D-ribose, and a new diaminohexose, named paramose, linked glycosidically (106). Haskell and Hanessian (107)

showed paramose to be identical with neosamine B, 2,6-diamino-2,6-
dideoxy-L-idose (99). Paromomycin has the structure shown in I (28).

(3) Zygomycin.—Zygomycin is also an antibiotic complex and was discovered by Horii and associates (108). Two diamino hexoses were

isolated from a hydrolyzate of zygomycin A. One of the diamino hexoses, which occurs in the zygomycin A fraction (109), is identical
Neomycin B \( (R = H, R' = R'' = CH_2NH_2) \)

Neomycin C \( (R = R'' = CH_2NH_2, R' = H) \)

Paromomycin I \( (R = H, R' = CH_2NH_2, R'' = CH_2OH) \)

Paromomycin II \( (R = CH_2NH_2, R' = H, R'' = CH_2OH) \)
with neosamine C, 2,6-diamino-2,6-dideoxy-D-glucose. The other diamino hexose, isolated from zygomycin A, fraction (109) was identical with neosamine B, paramose, 2,6-diamino-2,6-dideoxy-L-idose (99).

(4) Sharon and Jeanloz (110) isolated, from acid hydrolyzates of a polysaccharide of Bacillus subtilis, a diamino hexose. They proposed the structure 4-acetamido-2-amino-2,4,6-trideoxyhexose on the basis of color reactions, periodate studies, and infrared spectra. Later degradation studies (111) showed this to be 4-acetamido-2-amino-2,4,6-trideoxy-L-altrose.


Syntheses

2,5-Diamino-1,4;3,6-dianhydro-2,5-dideoxyhexitols (112).—These were prepared by heating 1,4;3,6-dianhydro-2,5-bis-O-(p-tolylsulfonyl)-D-mannitol and 1,4;3,6-dianhydro-2,5-bis-O-(p-tolylsulfonyl)-D-glucitol with methanolic ammonia under pressure. The configuration of these diamino hexitols was not verified. As suggested by Cope and Shen (45), these diamino hexitols have been assumed to have L-ido and L-gulo (D-gluco) configurations.

1,4:3,6-Dianhydro-2,5-dideoxy-2,5-bis(dimethylamino)-D-glucitol and L-iditol (45).—Heating 1,4:3,6-dianhydro-2,5-bis-O-(p-tolylsulfonyl)-D-glucitol and 1,4:3,6-dianhydro-2,5-bis-O-(p-tolylsulfonyl)-D-mannitol with anhydrous dimethylamine in tetrahydrofuran gave the title glucitol and iditol compounds, respectively.

1,2-Diamino-1,2-dideoxy-D-glucitol and D-mannitol (113).—The


D-glucitol compound was prepared by hydrogenation of 2-amino-2-déoxy-D-glucose phenylhydrazone with Raney nickel catalyst and was isolated as its crystalline bis(salicylaldehyde Schiff base) in low yield, which was identical with the product obtained by the hydrogenation of 2-amino-2-deoxy-D-glucosoxime(hydrochloride) (114,115) with palladium-

(114) R. Breuer, Ber., 31, 2193 (1898).


charcoal catalyst. The D-mannitol compound was isolated from the reduction products of D-arabino-hexosulose bis(phenylhydrazone) as its crystalline bis(salicylaldehyde Schiff base) derivative in low yield. Recently (116), both the title compounds have been isolated


as their bis(salicylaldehyde Schiff base) derivatives by catalytic
hydrogenation of D-arabino-hexosulose bis(phenylhydrazone) with Raney nickel in alkaline solution in better yield. The D-mannitol compound has been made (117) by the alternate route of reduction of D-arabino-


hexosulose l-(N-methylphenylhydrazone)2-oxime and the D-glucitol compound by a modification of the procedure of Wolfrom and co-workers (113).

1,2-Diamino-1,2-dideoxy-D-xylitol and D-lyxitol (116), D-three-
pentosulose bis(phenylhydrazone) on catalytic hydrogenation with Raney nickel in alkaline solution produced the two isomeric diamino alditalos which were isolated as their bis(salicylidene) derivatives. Catalytic hydrogenation of 2-amino-2-deoxy-D-lyxose phenylhydrazone and 2-amino-2-deoxylyxose oxime hydrochloride also produced the diaminolyxitol.

3,5-Diamino-3,5-dideoxy-1,2-0-isopropylidene-a-D-ribofuranose (54), This was obtained by Wolfrom and co-workers in 1959 as the bis(p-toluenesulfonate) salt from the hydrazinolysis of 1,2-0-isopropylidene-3,5-bis-0-(p-tolylsulfonyl)-a-D-xylofuranose and subsequent reduction of the product with Raney nickel catalyst.

1,3:2,4:Di-0-benzylidene-5,6-dideoxy-5,6-dipiperidino-D-glucitol (118). This compound was obtained by displacement of sulfonate ester


groups of 1,2:3,4-di-0-benzylidene-5,6-bis-0-(p-tolylsulfonyl)-L-iditol
with piperidine with inversion at C-5. Hydrolysis gave 5,6-dideoxy-5,6-dipiperidino-D-glucitol.

Derivatives of 1,6-diamino-1,6-dideoxyhexitols.—The title compounds with the D-manno- (119-121), D-gluco- (121), D-galacto- (121) and L-idoo (121) configurations were prepared by treating the appropriate 1,2,5,6-dianhydro or the 1,6-disubstituted sulfonate ester derivatives with 2-aminoethanol or 2,2-iminodietanol.

2,3-Diamino-2,3-dideoxy-D-mannose derivatives.—Treatment of methyl 2-azido-4,6-O-benzylidene-2-dideoxy-3-O-(p-tolylsulfanyl)-D-altroside with sodium azide in N,N-dimethylformamide and dioxane gave, according to Guthrie and Murphy (122), methyl 2,3-diazido-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside. Reduction of the diazido compound afforded methyl 2,3-diamino-4,6-O-benzylidene-2,3-dideoxy-α-D-mannoside, characterized as the crystalline dicetamido compound.

Derivatives of 2,3-diamino-2,3-dideoxy-D-altrose and D-glucose (123).—Methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-epimino-α-D-mannoside


and the corresponding alloside were opened with azide ion to give methyl 2-amino-3-azido-4,6-O-benzylidene-2,3-dideoxy-α-D-altroside and methyl 3-amino-2-azido-4,6-O-benzylidene-2,3-dideoxy-α-D-altroside, respectively (trans-diauxial products). They were hydrogenated to the same 2,3-diamino-2,3-dideoxy-D-altrose derivative. Opening of the substituted epimines followed the same pattern with the exception of the N-benzoylepimino-alloside which opened diequatorially to give 3-azido-2-benzamido-4,6-O-benzylidene-2,3-dideoxy-α-D-glucoside which on hydrogenation produced the 2,3-diamino-2,3-dideoxy-D-glucose derivative. On the other hand, treatment of the N-benzoylepimino-mannoside with sodium azide gave a mixture of methyl 3-azido-2-benzamido-4,6-O-benzylidene-2,3-dideoxy-α-D-altroside (trans-diauxial product) and methyl 4,6-O-benzylidene-2,3-dideoxy-3,2-(2-phenyl-1-oxa-3-azaprop-2-eno)-α-D-mannoside.

The reason for the anomalous diequatorial opening in one case only is not clear. As the N-benzoylepimino-mannoside opens normally, the mere presence of the benzoyl group cannot account for it. It is possible that both N-benzoylepimino-glycosides react through similar oxazolinium ion intermediates. Electron withdrawal by the inductive effect of the two oxygen atoms bound to C-1 causes the electron density at C-3 to be greater than that at C-2. Thus in an intramolecular rearrangement of either epimine derivative, the C-3—N bond should be more easily broken than the C-2—N bond. In such a mechanism, the PhCO group can readily resonance-stabilize the necessary ionic intermediate, in none of the other systems studied is this stabilization available.
2,4-Diamino-2,4-dideoxy-D-glucose derivatives (124).—Reaction


of 1,6-anhydro-2,4-bis-O-(p-tolylsulfonyl)-β-D-glucopyranose with saturated methanolic ammonia resulted in trans-diaxial opening of the monoepoxide ring to give predominantly 2,4-diamino-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose isolated as the fully acetylated derivative. The formation of this diamino product proceeded by the monoepoxide intermediate, 1,6:3,4-dianhydro-2-O-(p-tolylsulfonyl)-β-D-galactopyranose, while the other intermediate, 1,6:2,3-dianhydro-4-O-(p-tolylsulfonyl)-β-D-mannopyranose, appeared unfavorable. The D-glucopyranose configuration of 2,4-diacetamido-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose was confirmed by synthesis from 2-acetamido-1,6-anhydro-3-O-benzoyl-4-O-(methylsulfonyl)-β-D-galactopyranose with sodium azide, followed by hydrogenation and N-acetylation. Opening of the 1,6-anhydro ring of the diacetamido sugar was effected by acetylation, the crystalline 2,4-diacetamido-2,4-dideoxy-α-D-glucopyranose was obtained.

1,6-Diamino-1,6-dideoxy-2,3-O-isopropylidene-α-L-sorbofuranose (125).—This was obtained as a sirup on treatment of 1,4-anhydro-2,3-O-isopropylidene-6-O-(p-tolylsulfonyl)-α-L-sorbofuranose and 2,3-O-isopropylidene-1,6-bis-O-(p-tolylsulfonyl)-α-L-sorbofuranose with liquid ammonia.

Methyl 4,6-diamino-4,6-dideoxy-α-D-galactopyranoside (126).


Reaction of the 2,3-diaceitate or the 2,3-dibенzoate of methyl 4,6-bis-O-(methylsulfonyl)-α-D-glucopyranoside with sodium azide gave methyl 4,6-diazido-4,6-dideoxy-D-galactoside. This product was converted into a syrupy methyl 4,6-diamino-4,6-dideoxy-α-D-galactopyranoside characterized as the crystalline diacetamido derivative.

N-Derivatives of methyl 2,3-diamino-4,6-O-benzylidene-2,3-dideoxy-α-D-allopyranoside.—This type of cis-2,3-diamino sugar was obtained by Baker and Neilson by utilization of urea (127) or guanidine (128) neighboring group participation.


(128) Ibid., 1603 (1964).

Derivatives of 3,6-diamino-3,6-dideoxy-D-altrose (84,85).—These compounds were made by Wolf from and co-workers. Hydrazinolysis of methyl 2,6-bis-O-(methylsulfonyl)-α-D-glucopyranoside, followed by reduction, gave methyl 3,6-diamino-3,6-dideoxy-α-D-altropyranoside, isolable in high yield as the N,N'-diacetyl or N,N'-(2,4-dinitrophenyl) derivatives. The structure and stereochemistry of the products were proved by a sequence of degradation reactions and by comparison of the products with derivatives of known α-amino acids. 3,6-Diacetamido-3,6-dideoxy-D-altrose was prepared by way of 3,6-diacetamido-3,6-dideoxy-D-altrose diethyl dithiocetal.
3,6-Diamino-3,6-dideoxy-D-idose (76).—This diamino sugar was obtained by Hanessian and Haskell from methyl 2,6-bis-0-(p-tolyl-sulfonyl)-α-D-galactopyranoside. It was treated with sodium azide in dimethyl sulfoxide to give the corresponding 6-azido compound. Treatment with alcoholic sodium hydroxide then afforded crystalline methyl 2,3-anhydro-6-azido-6-deoxy-α-D-talopyranoside. The anhydro ring was opened by sodium azide to give methyl 3,6-diazido-3,6-dideoxy-α-D-idopyranoside as a major product. Reduction gave 3,6-diamino-3,6-dideoxy-D-idose dihydrochloride. Evidence in support of the ido configuration was based on physical data, degradation and color reactions.

Derivatives of 5,6-diamino-5,6-dideoxy-D-glucose and L-idose (129).—Derivatives of 5,6-diamino-5,6-dideoxy-D-glucose and L-idose


were obtained by blocking the 1, 2 and 3 positions of α-D-glucofuranose, cleaving the 5,6-glycol with periodate, condensing the generated carbonyl with nitromethane, base catalyzed elimination, addition of ammonia to the nitroolefin, and reduction of the nitro group. When attempts were made to obtain the free 5,6-diamino sugars by acid hydrolysis of the nitroamides or the reduced products, three moles of water were spontaneously eliminated and a pyridine derivative was obtained. Obviously a ring nitrogen species sugar was involved as an intermediate.

2,6-Diamino-2,6-dideoxy-β-D-glucose (130).—2-Acetamido-1,3,4—

tri-O-acetyl-6-azido-2,6-dideoxy-\(\beta\)-D-glucopyranose was prepared from 2-acetamido-1,3,4-tri-O-acetyl-2-deoxy-\(\beta\)-(p-tolylsulfonyl)-D-glucopyranose and sodium azide in dimethyl sulfoxide. Reduction and hydrolysis gave 2,6-diamino-2,6-dideoxy-\(\beta\)-D-glucose dihydrochloride.

\[
2,6\text{-Diamino-2,6-dideoxy-\(\alpha\)-D-glucose}
\]

Reduction of benzyl 3,4-di-O-acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-\(\alpha\)-D-glucopyranosidurononitrile by hydrogenation with platinum catalyst in acetic anhydride gave (Weidmann and Zimmerman, 101,102), benzyl 6-acetamido-3,4-di-O-acetyl-2-[(benzyloxycarbonyl)amino]-2,6-dideoxy-\(\alpha\)-D-glucopyranoside. Hydrogenolysis with palladium black in methanolic hydrogen chloride gave the dihydrochloride which on acid hydrolysis gave 2,6-diamino-2,6-dideoxy-D-glucose whose physical constants were identical with those of neosamine C, a hydrolytic fragment of the antibiotic neomycin C. Weidmann and Zimmerman (131) also prepared di-\(\pi\)-substituted derivatives using the same starting material. Hydrogenation of benzyl 3,4-di-O-acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-\(\alpha\)-D-glucopyranosidurononitrile in acetic acid over palladium, and adding hydrochloric acid, gave benzyl 3,4-di-O-acetyl-2,6-diamino-2,6-dideoxy-\(\alpha\)-D-glucopyranoside dihydrochloride. Similarly, addition of benzyl chloroformate gave benzyl 3,4-di-O-acetyl-2,6-bis[(benzyloxycarbonyl)amino]-2,6-dideoxy-\(\alpha\)-D-glucopyranoside while the addition of benzoyl chloride gave benzyl 3,4-di-O-acetyl-2,6-dibenzamido-2,6-dideoxy-\(\alpha\)-D-glucopyranoside. 2-Deacetylation of the former compound was achieved by refluxing with sodium methoxide in methanol. Methanolysis in the presence of hydrochloric acid gave benzyl 2,6-diamino-2,6-dideoxy-\(\alpha\)-D-glucopyranoside.
dihydrochloride. O-Deacetylation of benzyl 3,4-di-O-acetyl-2,6-
dibenzamido-2,6-dideoxy-α-D-glucopyranoside gave benzyl 2,6-dibenzamido-
2,6-dideoxy-α-D-glucopyranoside.

Rinehart and co-workers (102) also prepared 2,6-diamino-2,6-
dideoxy-D-glucose starting with the known methyl 2-acetamido-2-deoxy-
α-D-glucopyranoside. It was converted into the 6-O-(p-tolylsulfonyl)
compound which was heated with saturated methanolic ammonia to give the
6-amino compound, isolated as a crystalline methyl 2,6-diacetamido-
2,6-dideoxy-α-D-glucopyranoside. Hydrolysis gave 2,6-diamino-2,6-
dideoxy-D-glucose dihydrochloride which was identical with neosamine C.

Weidmann and Zimmerman (132) have reported benzyl 2,6-diamino-


2,6-dideoxy-α-D-glucopyranoside dihydrochloride made by the hydrogena-
tion of benzyl 2-[(benzyloxy carbonyl)amino]-2-deoxy-α-D-glucopyra-
nonitride with palladium black catalyst in the presence of
methanolic hydrogen chloride. They have also reported 2,6-diamino-
2,6-dideoxy-D-glucose dihydrochloride made by the hydrogenation of
benzyl 2-[(benzyloxy carbonyl)amino]-2-deoxy-α-D-glucopyranosiduro-
nitride with 10% palladium on carbon in methanolic hydrogen chloride
solution and by further hydrogenation in aqueous solution in the
presence of 10% palladium on carbon.

2,6-Diamino-2,6-dideoxy-L-idose (103,104).—Heating methyl
2-benzamido-2-deoxy-3-O-methyl-5,6-bis-O-(methylsulfonyl)-α-D-
glucofuranoside with sodium azide in \textit{N}_{2}N\textit{-dimethylformamide gave the
6-azido compound. Inversion of this sugar at C-5 was achieved by
refluxing with sodium acetate in 95% ethanol to give methyl 2,6-dibenzamido-2,6-dideoxy-3-0-methyl-α-L-idofuranose. Demethylation with boron trichloride and hydrolysis gave 2,6-diamino-2,6-dideoxy-L-idose which was shown to be identical with natural neosamine B and paromosine. N-Acetylation gave crystalline 2,6-diacetamido-2,6-dideoxy-L-idose.

2,3-Diamino-2,3-dideoxy-D-allose and 2,3-diamino-2,3-dideoxy-α-D-glucose (133).—These two diamino sugars were prepared by Meyer zu Reckendorf. Heating methyl 2-benzamido-4,6-0-benzylidene-2-deoxy-3-0-(methylsulfonyl)-β-D-glucopyranoside with sodium azide in dimethyl sulfoxide gave a mixture of 3-azido-2-benzamido-4,6-0-benzylidene-2,3-dideoxy-β-D-allopyranoside and methyl 4,6-0-benzylidene-2,3-dideoxy-3,2-(2-phenyl-1-oxa-3-azaprop-2-eno)-β-D-allopyranoside. The latter was separated from the former as the hydrochloride. Reduction of the 3-azido sugar with hydrogen and palladium on carbon and hydrolysis gave 2,3-diamino-2,3-dideoxy-D-allose dihydrochloride. The 2,3-diacetamido- and 2,3-bis(2,4-dinitroanilino)-derivatives were also isolated. Heating methyl 2-benzamido-4,6-0-benzylidene-2-deoxy-3-0-(methylsulfonfyl)-β-D-allopyranoside with sodium azide in acetone-water gave the 3-azido derivative with the β-D-gluco-configuration. Catalytic hydrogenation and acid hydrolysis provided crystalline 2,3-diamino-2,3-dideoxy-α-D-glucopyranose.

2,6-Diamino-2,6-dideoxy-α-D-allose.—This diamino sugar was synthesized by Reckendorf (133) as follows. Reaction of methyl 2-
2-benzamido-2-deoxy-3-O-(methylsulfonyl)-6-O-(p-tolylsulfonyl)-8-D-glucopyranoside with sodium azide in dimethyl sulfoxide and subsequent chromatography on an alumina column yielded methyl 3,6-diazido-2-benzamido-2,3,6-trideoxy-8-D-allopyranoside, methyl 3,4-anhydro-6-azido-2-benzamido-2,6-dideoxy-8-D-allopyranoside and methyl 6-azido-2,3,6-trideoxy-3,2-(2-phenyl-1-oxa-3-azaprop-2-enoyl)-8-D-allopyranoside. The last compound on catalytic hydrogenation and subsequent benzoylation produced methyl 6-benzamido-2,3,6-trideoxy-3,2-(2-phenyl-1-oxa-3-azaprop-2-enoyl)-8-D-allopyranoside. This compound, when heated with methanolic hydrogen chloride, gave methyl 2,6-dibenzamido-2,6-dideoxy-8-D-allopyranoside. Hydrolysis of this provided 2,6-diamino-2,6-dideoxy-8-D-allose dihydrochloride.

Treatment of methyl 2-benzamido-2-deoxy-3-O-(methylsulfonyl)-6-O-(p-tolylsulfonyl)-8-D-glucopyranoside with sodium azide in acetonewater produced methyl 6-azido-2-benzamido-2,6-dideoxy-3-O-(methylsulfonyl)-8-D-glucopyranoside which on catalytic hydrogenation yielded methyl 6-amino-2-benzamido-2,6-dideoxy-3-O-(methylsulfonyl)-8-D-glucopyranoside. This compound on treatment with anhydrous sodium acetate in absolute ethanol and N-benzoylation produced a syrup which on chromatographic separation (thin layer) produced methyl 6-benzamido-2,3,6-trideoxy-3,2-(2-phenyl-1-oxa-3-azaprop-2-enoyl)-8-D-allopyranoside. This compound on treatment with methanolic hydrogen chloride produced methyl 2,6-benzamido-2,6-dideoxy-8-D-allopyranoside which on acid hydrolysis gave 2,6-diamino-2,6-dideoxy-8-D-allose dihydrochloride.

Zimmerman and co-workers (134,135) have made the same diamino

(134) P. H. Gross, K. Brendel, and H. K. Zimmerman, Jr., Naturwiss., 51, 509 (1964)
sugar from a 2-benzamido-2-deoxy-D-glucose derivative by inversion at C-3 with thionyl chloride to yield a 2-benzamido-2-deoxyallose derivative. The 6-amino group was later introduced by a route involving replacement of the 6-0-(methylsulfonyl) group by sodium azide followed by hydrogenation. Acid hydrolysis, finally, yielded 2,6-diamino-2,6-dideoxy-D-allose dihydrochloride.

Physical constants reported by Zimmerman and co-workers, however, disagree with those reported by Reckendorf.

2,6-Diamino-2,6-dideoxy-D-gulose (136,137).—This was prepared by Zimmerman and co-workers through an oxazolidone intermediate. Heating benzyl 3,4-anhydro-2-[(benzyloxy carbonyl)amino]-2-deoxy-6-0-(methylsulfonyl)-α-D-galactopyranoside in 50% acetic acid led to a stero-specific cleavage of the anhydro ring with participation of the neighboring benzyloxy carbonyl to form an oxazolidone ring system (138). The product, benzyl 4-0-acetyl-6-0-(methylsulfonyl)-α-D-glucopyranosid-(2,3-d)oxazolid-2′-one was isolated by acetylation. Treatment of this with sodium azide in dimethyl sulfoxide gave the 6-azido compound which was converted by hydrogenation and hydrolysis...
into the compound 2,6-diamino-2,6-dideoxy-D-gulose dihydrochloride.

2,6-Diamino-2,6-dideoxy-D-galactose.—This compound was prepared (139) starting from methyl 2-benzamido-2-deoxy-3-O-methyl-4,6-di-O-(methylsulfonyl)-p-D-glucopyranoside. Heating with sodium azide in dimethyl sulfoxide gave a 6-azido derivative which was then converted to the 6-benzamido analog by reduction and benzoylation. Heating with sodium methoxide caused the back side attack of the 6-benzamido group on the C-4 methylsulfonyl group with inversion to give an oxazoline derivative having a D-galacto configuration. Refluxing with methanolic hydrogen chloride followed by benzoylation gave methyl 2,6-dibenzamido-4-O-benzoyl-2,6-dideoxy-3-O-methyl-6-D-galactopyranoside. 0-Demethylation with boron trichloride followed by hydrolysis produced 2,6-diamino-2,6-dideoxy-6-D-galactose dihydrochloride.

Another method (140) for synthesis of the title compound was the reduction of the nitrile derivative. Benzyl 3,4-di-O-acetyl-2-[(benzyloxy carbonyl)amino]-2-deoxy-p-D-galactopyranosiduronam ide was dehydrated with triphenyl phosphine dibromide to give a urononitrile. Hydrogenation, peracetylation and acid hydrolysis afforded 2,6-diamino-2,6-dideoxy-D-galactose dihydrochloride.

3,4-\(\omega\)-isopropylidene-2-\(\omega\)-(methylsulfonyl)-\(\alpha\)-D-galactopyranosidurononitrile was converted into methyl 6-acetamido-6-deoxy-2-\(\omega\)-(methylsulfonyl)-\(\alpha\)-D-galactopyranoside in three steps followed by treatment with sodium methoxide in methanol to yield methyl 6-acetamido-2,3-anhydro-6-deoxy-\(\alpha\)-D-talopyranoside. The latter compound was obtained in similar yield from methyl 3,4-\(\omega\)-isopropylidene-2-\(\omega\)-(p-tolylsulfonyl)-\(\alpha\)-D-galactopyranosidurononitrile (142). Ammonolysis of the 2,3-epoxide, with partial N-deacetylation, produced a mixture of methyl 3,6-diamino-3,6-dideoxy- and methyl 6-acetamido-3-amino-3,6-dideoxy-\(\alpha\)-D-idoside which on acetylation produced the title compound.

4,5-Diacetamido-4,5-dideoxy-L-xylopyranose.—The title compound has been made by Wolfrom and co-workers (143) as follows. 1,2-Diamino-

1,2-dideoxy-D-glucitol dihydrochloride (I) was converted into 1,2-diacetamido-1,2-dideoxy-D-glucitol (II). With cupric sulfate and sulfuric acid as catalysts, acetonation of II gave 1,2-diacetamido-1,2-dideoxy-5,6-\(\omega\)-isopropylidene-D-glucitol (III), whose structure was established by periodate oxidation. With zinc chloride, phosphorus
pentaoxide and phosphoric acid as catalysts, \(1,2\)-diacetamido-\(1,2\)-dideoxy-\(3,4\)-\(6\)-di-\(\text{o}\)-isopropylidene-D-glucitol (IV) was obtained. The \(5,6\)-\(\text{o}\)-isopropylidene group could be selectively removed by acid to give \(1,2\)-diacetamido-\(1,2\)-dideoxy-\(3,4\)-\(\text{\text{o}}\)-isopropylidene-D-glucitol (V) whose structure was likewise established by periodate oxidation. Preparative glycol cleavage of V with periodate afforded \(4,5\)-diacetamido-\(4,5\)-dideoxy-\(2,3\)-\(\text{o}\)-isopropylidene-aldehydo-L-xylose (VI). Paper chromatography of the products of acid hydrolysis of VI indicated a major and a minor component. The major product crystallized from the mixture and was formulated as \(4,5\)-diacetamido-\(4,5\)-dideoxy-L-xylopyranose (VII) by n.m.r. data.

\[4,5\text{-Diacetamido-}4,5\text{-dideoxy-L-xylopyranose and 4-acetamido-}4,5\text{-dideoxy-D-xylofuranose (144).—}
\]

(144) S. Hanessian, ibid., 1, 178 (1965).

arabinose diethyl dithiocetal afforded the \(2,3\)-\(\text{o}\)-isopropylidene derivative which was subsequently converted to the \(4,5\)-\(\text{\text{o}}\)-(methyilsulfonyl) derivative. Treatment of the latter with sodium azide in N,N-dimethylformamide afforded predominantly \(4,5\)-diazido-\(4,5\)-dideoxy-\(2,3\)-\(\text{o}\)-isopropylidene-L-xylose diethyl dithiocetal (I). A second product, \(4,5\)-diazido-\(4,5\)-dideoxy-\(2,3\)-\(\text{o}\)-isopropylidene-D-arabinose diethyl dithiocetal (II), formed in an approximate ratio of 1:60 with respect to I was also isolated. Reduction of I with lithium aluminum hydride, followed by N-acetylation, afforded the corresponding \(4,5\)-diacetamido-\(4,5\)-dideoxy-L-xylose diethyl dithiocetal (IV).
Demercaptylation of IV afforded a mixture of the two title compounds as sirups. They remained as sirups even when they were separated by paper chromatography.
STATEMENT OF THE PROBLEM

The object of this research was to provide reference compounds for comparison with possible fragmentation products obtained from aminated amylose (77) in structural studies by degradative methods. Possible routes for amination of a 2,6-bis-O-(p-tolylsulfonyl)-α-D-glucopyranose unit in a (1 → 4)-linked chain have already been discussed (See Development of the Problem). Such an aminated polymer is expected to produce, on acid hydrolysis, 3,6-diamino-3,6-dideoxy-D-altrose, 2,6-diaminohex-2,6-dideoxy-D-mannose, and 2-amino-3,6-anhydro-2-deoxy-D-mannose in various proportions. The synthesis of 3,6-diamino-3,6-dideoxy-D-altrose has been discussed elsewhere (84,85). This thesis purports to describe syntheses of 2,6-diamino-2,6-dideoxy-D-mannose and 2-amino-3,6-anhydro-2-deoxy-D-mannose and their various derivatives.

This thesis also purports to describe briefly the various unsuccessful attempts made to introduce an amino group on C-2 of 3,6-anhydroamylose and degradative experiments on N-acetyl aminated amylose (77).
Graphical Summary (Part I)

I. NaOMe
   CH₂OH
   OH
   HO
   NH₃Cl
   NaOMe
   Ac₂O
   CH₂OH
   OH
   HO
   NHAc

II. PhOH, ZnCl₂
    CH₂OAc
    O
    O
    AcNH
    OAc
   AcNH
   OAc
   CH₂OAc
   O
   O
   AcNH
   OAc
   MeOH
   CH₂OH
   OH
   HO
   AcNH
   OAc
   Ph
   OPh

III. TsCl / Py
     CH₂OH
     OH
     HO
     AcNH
     OAc
     CH₂OH
     OH
     HO
     AcNH
     OAc
     Ph

IV. NaN₃
    CH₂OTs
    O
    O
    AcNH
    RO
    O
    CH₂OTs
    O
    O
    AcNH
    RO
    O
    CH₂NH₂Cl

V. Pd-C / H₂
   CH₂N₃
   O
   O
   AcNH
   OAc
   CH₂N₃
   O
   O
   AcNH
   OAc
   CH₂NH₂Cl

VI. Pd-C / H₂
    CH₂NH₂Cl
    O
    O
    AcNH
    OAc
    CH₂NH₂Cl
    O
    O
    AcNH
    OAc
    CH₂NH₂Cl

VII. Acetone-H₂O
     CH₂OTs
     O
     O
     AcNH
     RO
     O
     CH₂OTs
     O
     O
     AcNH
     RO
     O
     CH₂NH₂Cl

VIII. Pd-C / H₂
      CH₂N₃
      O
      O
      AcNH
      OAc
      CH₂N₃
      O
      O
      AcNH
      OAc
      CH₂NH₂Cl

IX. Pd-C / H₂
    CH₂N₃
    O
    O
    AcNH
    OAc
    CH₂N₃
    O
    O
    AcNH
    OAc
    CH₂NH₂Cl

X. 6N HCl
    CH₂NH₂Cl
    O
    O
    AcNH
    OAc
    CH₂NH₂Cl
    O
    O
    AcNH
    OAc
    CH₂NH₂Cl

1. Py / Ac₂O
2. NaOAc / Ac₂O
1. TsCl / Py
2. Py / Ac₂O
1. Pd-C / H₂
2. 6N HCl
1. Pd-C / H₂
2. Py / Ac₂O
6N HCl
1. NaOH
EtOH-H₂O
or
2. NaOAc/ EtOH

Py / Ac₂O

EtSH/HCl

Py / Ac₂O

CH(SEt)₂

CH₂

AcN

OAc

CH₂

AcN

OAc

CH₂

AcN

OAc

CH₂

AcN

OAc

CH₂

AcN

OAc

CH₂

AcN

OAc
DISCUSSION OF RESULTS

Part I

Part I is concerned with the synthesis of 2,6-diamino-2,6-dideoxy-D-mannose dihydrochloride and of 2-amino-3,6-anhydro-2-deoxy-D-mannose hydrochloride.

Synthesis of 2,6-Diamino-2,6-dideoxy-
D-mannose Dihydrochloride

In order to synthesize 2,6-diamino-2,6-dideoxy-D-mannose by a stereochmically unambiguous route it was decided to start with 2-amino-2-deoxy-D-mannose and to introduce the second amino group exclusively on C-6 by utilizing the difference of reactivity between primary and secondary hydroxyl groups.

A few different ways to synthesize 2-amino-2-deoxy-D-mannose have been reported in the literature. Levene (145) first synthesized it by reducing 2-amino-2-deoxy-D-mannonolactone with sodium amalgam. Kuhn and Bister (146) isolated 2-amino-2-deoxy-D-mannose hydrochloride in a 1% yield from the mother liquor of the reaction to synthesize 2-amino-2-deoxy-D-glucose hydrochloride by catalytic hydrogenation.

of 2-benzylamino-2-deoxy-D-glucononitrile. O'Neill (147) synthesized


2-amino-2-deoxy-D-mannose hydrochloride as follows. The addition of
ammonia to the ethylenic bond of D-arabino-3,4,5,6-tetracetoxy-1-
nitro-1-hexene was stereospecific and formed a crystalline product,
2-acetamido-1,2-dideoxy-1-nitro-D-mannitol. Application of the
Nef reaction to this compound gave 2-amino-2-deoxy-D-mannose, which
was isolated as the crystalline pentaacetate and further character-
ized as the hydrochloride by deacetylation with hydrochloric acid.
In the deacetylation of the pentaacetate with barium methoxide,
however, an abnormal epimerization occurred to yield 2-acetamido-2-
deoxy-D-glucose.

Sowden and Oftedahl (148) synthesized 2-amino-2-deoxy-D-mannose

(148) J. C. Sowden and M. L. Oftedahl, J. Am. Chem. Soc., 82,
2303 (1960).

hydrochloride by essentially following O'Neill's route (147). But,
contrary to the findings of O'Neill, who concluded that the addition
of ammonia to D-arabino-3,4,5,6-tetracetoxy-1-nitro-1-hexene was
stereospecific and gives only the D-manno isomer, they found rather
that the addition was simply stereoselective and that the yield of
D-manno:D-gluco isomer was approximately 6:1. The epimeric substances
were, however, readily separated by fractional crystallization from
absolute ethanol. Application of the Nef reaction, using hydrochloric
acid rather than sulfuric acid, to 2-acetamido-1,2-dideoxy-1-nitro-D-
mannitol, followed by heating to hydrolyze the amide linkage yielded 2-amino-2-deoxy-D-mannose hydrochloride.

Satoh and Kiyomoto (149) have synthesized 2-acetamido-2-deoxy-


D-mannose by a modification of the procedure of Sowden and Ofstedahl (148). Reaction of 1-nitro-1-deoxy-D-mannitol pentaacetate with methanolic ammonia produced a mixture of 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol and 2-acetamido-1,2-dideoxy-1-nitro-D-glucitol with the former predominating. They were separated by fractional crystallization from absolute ethanol. Application of the Nef reaction, using the barium nitronium salt and sulfuric acid, to 2-acetamido-

1,2-dideoxy-1-nitro-D-mannitol yielded 2-acetamido-2-deoxy-D-mannose which crystallized as the monohydrate.

There are a few reports (150-152) in the literature on alkaline epimerization of 2-acetamido-2-deoxy-D-glucose to 2-acetamido-2-deoxy-


d-mannose. The two amino sugars can be separated either by fractional crystallization (151,152) or 2-acetamido-2-deoxy-D-glucose can be removed from the mixture, leaving 2-acetamido-2-deoxy-D-mannose in
solution, by using *E. coli* strain B, adapted to grow on 2-acetamido-2-deoxy-D-glucose as the sole carbon source (150).

The above alkaline epimerization can be regarded as similar to the Lobry de Bruyn-Alberda von Ekenstein isomerization of reducing sugars in dilute base, which proceeds through intermediary enediols (153). But reaction of 2-acetamido-2-deoxyaldoses is not complicated by the formation of 2-ketoses or 2-ketimines, in contrast to that of normal adoses and 2-amino-2-deoxyaldoses. Epimerization of 2-acetamido-2-deoxy-D-glucose to 2-acetamido-2-deoxy-D-mannose takes place in weakly alkaline solution; under such condition unsubstituted monosaccharides are not epimerized. The rate-determining step in this process is the rate of epimerization of the C-2 hydrogen atom, which leads to the enolate ion II (154).


The electrophilic inductive effect of the acetamido group is greater than that of the hydroxyl group by virtue of the fractional positive charge on the nitrogen atom which is produced by resonance. Thus, the 2-acetamido-2-deoxy-aldoses will epimerize through the acyclic form (I) and the electron-attracting effect of the acetamido group will accelerate the ionization of the 2-hydrogen atom and hence increase the rate of formation of the enolate anion (II), with consequent destruction of asymmetry at position 2 and later formation of the two epimers.

Although the rate of carbanion formation is not necessarily linearly correlated with the acidic dissociation constant of the ionizing proton, the idea is well substantiated that electrophilic substituents on a carbon atom to which a potentially acidic hydrogen is attached can increase the rate of carbanion formation.

With 2-acetamido-2-deoxyhexoses, although the epimerization will proceed through the acyclic forms, the resting state of the molecules will probably be the pyranoid ring form (155). Hence the proportion


of each epimer in the equilibrium mixture will be governed by the relative stabilities of their pyranose chain conformations.

In the present work 2-acetamido-2-deoxy-D-mannose was made by alkaline epimerization of 2-acetamido-2-deoxy-D-glucose according to Kuhn and Baschang (152).

2-Acetamido-2-deoxy-D-glucose (II) was made by selective
N-acetylation of 2-amino-2-deoxy-D-glucose hydrochloride (I) following the method described by Horton (156). 2-Acetamido-2-deoxy-D-glucose (II) was obtained in 94% yield. Epimerization of 2-acetamido-2-deoxy-D-glucose (II) by the procedure of Kuhn and Baschang (152) gave 2-acetamido-2-deoxy-D-mannose (III) in 12% yield. Most of the unchanged starting material could be recovered and was re-cycled in the epimerization procedure. The crystalline product was of 95% purity as determined by optical rotation and paper chromatography. 2-Acetamido-2-deoxy-D-mannose (III) was converted into 2-acetamido-tetra-O-acetyl-2-deoxy-β-D-mannopyranose (IV), in 56% yield, by heating with acetic anhydride and anhydrous sodium acetate. The same product was obtained, in approximately the same yield if the acetylation was performed with acetic anhydride-pyridine by the procedure of O'Neill (147) or Levene (157). The compound IV was then converted into its phenyl glycoside (V) by fusion with phenol.

The preparation of both phenyl α- and β-D-glycoside acetates, by fusing a fully acetylated sugar with phenol in the presence of an acidic catalyst, was first described by Helferich and Schmitz-Hillebrecht (158). When an acetylated reducing sugar is heated
with phenol in the presence of fused zinc chloride as catalyst, the formation of the \( \alpha-D \)-anomer is favored, while in the presence of \( p \)-toluenesulfonic acid as catalyst the formation of \( \beta-D \)-anomer is favored. Shishido (159) improved the yields of both \( \alpha \)- and \( \beta \)-forms by carrying out the reaction under reduced pressure and thus removing the acetic acid which was liberated. Hudson and co-workers (160) improved the yields still more by dissolving the fused zinc chloride in a mixture of acetic acid and acetic anhydride before adding it to the other components of the reaction mixture. Fujise and Yokoyama (161) fused the acetylated 2-amino-2-deoxy-D-glucose with various aromatic phenols in the presence of either zinc chloride or \( p \)-toluenesulfonic acid and obtained the corresponding acetylated aryl glycosides in very low yield. They assigned probable \( \beta-D \) configurations to them based on the sign of rotation. Zissis and Richtmyer (162) described the condensation of 


sedoheptulose hexaacetate and phenol, with zinc chloride as catalyst, as producing phenyl α-sedoheptuloside pentaacetate in 84% yield and phenyl β-sedoheptuloside in very low yield.

In the present work, fusion (160) of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-mannose (IV) with phenol and zinc chloride, dissolved in 19:1 acetic acid — acetic anhydride, produced phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-mannoside (V) in 65% yield. The same product was obtained in similar yield, if p-toluenesulfonic acid was used in place of zinc chloride in acetic acid — acetic anhydride. The anomic assignment of V was based on its high specific rotation (+74° in chloroform) and also on the nuclear magnetic resonance data of one of its derivatives (see below). O-Deacetylation of V with sodium methoxide in methanol gave crystalline phenyl 2-acetamido-2-deoxy-α-D-mannopyranoside (VI) in 85% yield. Unimolecular p-toluene-sulfonation of the latter compound gave the sirupy, chromatographically homogeneous phenyl 2-acetamido-2-deoxy-6-O-(p-tolylsulfonyl)-α-D-mannopyranoside (VII, R=H) in high yield, characterized as the crystalline, 3,4-diacetate (VII, R=Ac). Displacement of the p-tolylsulfonyloxy group from either (VII, R=H) or (VII, R=Ac) by treatment with sodium azide in aqueous acetone (65) at 100° gave sirupy phenyl 2-acetamido-6-azido-2,6-dideoxy-α-D-mannopyranoside (VIII, R=H) or the crystalline 3,4-diacetate (VIII, R=Ac), respectively, in good yield. Acetylation of the sirupy product VIII (R=H) gave crystalline VIII (R=Ac). Hydrogenolysis of the azido group over palladium-carbon, followed by acetylation with acetic anhydride in pyridine, converted VIII (R=Ac) into the crystalline phenyl 2,6-diacetamido-3,4-di-O-acetyl-2,6-dideoxy-α-D-mannoside (IX) in good yield. Final deblocking was achieved by
direct hydrolysis with 6 N hydrochloric acid, to give crystalline 2,6-diamino-2,6-dideoxy-2-D-mannose dihydrochloride (X), which melted with decomposition at 157° and showed an upward mutarotation. The diamino sugar (X) could also be obtained directly from sirupy phenyl 2-acetamido-6-azido-2,6-dideoxy-α-D-mannopyranoside (VIII, R=H), or its 3,4-diacetate (VIII, R=Ac) by hydrogenation and subsequent hydrolysis with 6 N hydrochloric acid.

While the results of this work were being prepared for publication (163,164) a paper appeared (165) in which the synthesis of 2,6-diamino-

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(165) W. Meyer zu Reckendorf, Ber., 98, 93 (1965).

2,6-dideoxy-D-mannose dihydrochloride (X) was described. The oxazoline derivative (i) prepared from methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-2-O-(methylsulfonyl)-α-D-altropyranoside (iii), was used in the
synthesis of 2,6-diamino-2,6-dideoxy-\(\beta\)-D-mannose dihydrochloride by way of the derivative (ii, \(R=N_2\), \(R'=\text{Ac}\)). A mixture of iii, potassium cyanide and \(N,N\)-dimethylformamide produced, on heating, 25\% of i together with 49\% of methyl \(N\)-benzoyl-4,6-O-benzylidene-2,3-dideoxy-2,3-imino-\(\alpha\)-D-mannopyranoside (iv). Refluxing iii with anhydrous sodium acetate in absolute ethanol gave 84\% of i. Refluxing of i with methanolic hydrogen chloride produced ii \((R=\text{OH}, R'=\text{H})\) in 52\% yield and this on unimolecular \(p\)-toluenesulfonation, followed by acetylation in pyridine, gave 48\% of ii \((R=\text{OTs}, R'=\text{Ac})\). Treatment of ii \((R=\text{OTs}, R'=\text{Ac})\) with sodium azide in dimethyl sulfoxide gave ii \((R=N_2, R'=\text{Ac})\) in 100\%, which on reduction with hydrogen in the presence of palladium-carbon and subsequent hydrolysis with 6\% hydrochloric acid gave 64\% of 2,6-diamino-2,6-dideoxy-D-mannose dihydrochloride, which melted with decomposition at 155° and showed an upward mutarotation. The di-\(N\)-acetyl derivative of this compound showed a melting point of 211-212°.

In a brief, preliminary communication appearing after that of ours, Zimmerman and co-workers (166) report the synthesis of 2,6-

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(166) P. H. Gross, K. Brendel, and H. K. Zimmerman, Jr., Naturwiss., 52, 185 (1965).

diamino-2,6-dideoxy-D-mannose dihydrochloride hemihydrate. They converted 2-acetamido-2-deoxy-D-mannopyranose into a sirupy ethyl 2-acetamido-2-deoxy-D-mannopyranoside. This compound, on unimolecular \(p\)-toluenesulfonation, displacement of the sulfonyloxy group with azide ion, catalytic hydrogenation and hydrolysis with hydrochloric
acid, produced 2,6-diamino-2,6-dideoxy-D-mannose dihydrochloride hemihydrate which showed a melting point of 130-140° and a downward mutarotation. The hemihydrate form was not established unequivocally in this communication.

_Synthesis of 2-Amino-3,6-anhydro-2-deoxy_D-mannose Hydrochloride_

The p-toluenesulfonyloxy group in methyl 3,6-anhydro-2-O-(p-tolylsulfonyl)-α-D-glucopyranoside could not be displaced by hydrazine even under very severe conditions (88). It was accordingly decided to prepare phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XI) from phenyl 2-acetamido-2-deoxy-6-O-(p-tolylsulfonyl)-α-D-mannopyranoside (VII, R=H) by intramolecular displacement of the 6-p-tolylsulfonyloxy group by the 3-oxygen. It was also interesting to determine whether the 2-nitrogen would attack on C-6 under the conditions of reaction.

Treatment of syrupy phenyl 2-acetamido-2-deoxy-6-O-(p-tolylsulfonyl)-α-D-mannopyranoside (VII, R=H) in absolute ethanol with N sodium hydroxide solution (167) at room temperature for 24 hours


produced crystalline phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XI) in 77% yield. The same compound (XI) was obtained in 65% yield when a mixture of VII (R=H) and anhydrous sodium acetate in absolute ethanol was heated under reflux for 36 hours. Treatment
of phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XI) with pyridine and acetic anhydride produced crystalline phenyl 2-acetamido-4-O-acetyl-3,6-anhydro-2-deoxy-α-D-mannoside (XII) in 85% yield. The n.m.r. spectrum of this compound in deuteriochloroform showed a singlet at \( \gamma 8.06 \) (3-protons), assigned to NAc (equatorial) and another singlet at \( \gamma 7.90 \) (3-protons), assigned to OAc (axial) (168,169). The anomic proton signal appeared as a doublet, \( \gamma 4.67 \), \( J_{1,2} 7.8 \text{ cps} \). The high \( J \) value confirmed the anomic assignment. A one-proton doublet at \( \gamma 3.44 \) (\( J 9.0 \text{ cps} \)), which disappeared on deuteration, was assigned to the NH proton. Hydrolysis of phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XII) with 6 N hydrochloric acid produced crystalline 2-amino-3,6-anhydro-2-deoxy-α-D-mannofuranose hydrochloride (XIII) in 81% yield. This substance had an indefinite melting point and did not mutarotate. Analysis of the n.m.r. spectrum of 2-amino-3,6-anhydro-2-deoxy-α-D-mannofuranose hydrochloride (XIII) in deuterium oxide, allowing sufficient time for complete exchange of acidic hydrogen atoms, indicated it to be a mixture of anomers and confirmed the furanose ring structure. The anomic proton signal appeared as a pair of doublets at \( \gamma 4.30 \) and \( 4.42 \), with relative intensities 3:1, \( J_{1,2} 5.4 \) and 4.2 cps respectively. A pair of multiplets at \( \gamma 5.03-5.42 \) and 5.49-5.72, corresponding to four protons were assigned to the ring protons at C-2.


C-3, C-4, and C-5. A multiplet at δ 5.92-6.33, corresponding to two protons, assigned to two protons at C-6.

The conversion of a pyranose ring into a furanose ring also follows logically from the fact that a 3.2.1-bicyclooctane ring system, as present in phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XI), is thermodynamically less stable than two cis-fused five membered rings, as present in 2-amino-2-deoxy-D-mannofuranose hydrochloride (XIII). It is known that in simple glycosides, the pyranoside form is relatively much more stable toward acid hydrolyzing agents than is the furanoside but the introduction of an oxygen bridge between C-3 and C-6 reverses this order of stability (46). Haworth and co-workers (170) have shown that methyl 3,6-anhydro-D-glucopyranoside is nearly instantaneously converted into the corresponding furanoside, without the loss of the glycosidic methyl group, in the presence of ethereal hydrogen chloride. The same transformation takes place with similar rapidity in the presence of methanolic hydrogen chloride. On similar treatment, methyl 3,6-anhydro-β-D-galactopyranoside produces 3,6-anhydro-β-D-galactose dimethyl acetal (46). In this case the closure of the furanoside ring is not possible because this would lead to the formation of two trans-fused five membered rings which would be thermodynamically less stable.

Valentin (171) found that methyl 3,6-anhydro-α-D-mannopyranoside,


on acid hydrolysis, produced crystalline 3,6-anhydro-\(\alpha\)-D-mannofuranoside which did not show any mutarotation.

Acetylation of 2-amino-3,6-anhydro-2-deoxy-\(\alpha\)-mannofuranose hydrochloride (XIII) with pyridine and acetic anhydride produced a sirupy 2-acetamido-1,5-di-\(\alpha\)-acetyl-3,6-anhydro-2-deoxy-\(\alpha\)-mannose (XIV) in 70% yield; this substance was chromatographically homogeneous.

Phenyl 2-acetamido-3,6-anhydro-2-deoxy-\(\alpha\)-D-mannopyranoside (XI), on reaction with ethanethiol and concentrated hydrochloric acid at 0°, produced sirupy 2-acetamido-3,6-anhydro-2-deoxy-\(\alpha\)-mannose diethyl dithioacetal (XV) in 92% yield; this substance was chromatographically homogeneous. Acetylation of XV with pyridine and acetic anhydride produced sirupy 2-acetamido-4,5-di-\(\alpha\)-acetyl-3,6-anhydro-2-deoxy-\(\alpha\)-mannose diethyl dithioacetal (XVI) in 93% yield; this substance was chromatographically pure. The n.m.r. spectrum of XVI showed a triplet at \(\gamma 8.73\) corresponding to \(\text{CH}_3\) (6 protons), a quintet at \(\gamma 7.40\) corresponding to \(\text{CH}_2\) (4 protons) and three three-proton singlets at \(\gamma 8.08, 8.05\), and 7.86 corresponding to three acetyl groups. A single proton doublet at \(\gamma 3.97\) (J 9.60 cps) was assigned to the NH proton since it disappeared on deuteration.

Attempted opening of the 3,6-anhydro ring in XI with boron trichloride in dichloromethane failed.
Part II

Part II is concerned with the attempted synthesis of 2-amino-3,6-anhydro-2-deoxyamylose and with the degradative experiments on N-acetyl aminated amylose.

**Attempted Synthesis of 2-Amino-3,6-anhydro-2-deoxyamylose**

In order to achieve amination of amylose exclusively at C-2, various attempts were made to displace the sulfonyloxy group of a 3,6-anhydro-2-0-sulfonylamylose by different nitrogen nucleophiles.

Bines and Whelan (172) have described a synthetic route for


3,6-anhydroamylose involving tritylation of amylose with trityl chloride (chlorotriphenyl methane), followed by benzoylation, detritylation, \( p \)-toluenesulfonation and final saponification. Whistler and Hirase (60) introduced the 3,6-anhydro ring into amylose by direct \( p \)-toluenesulfonation of amylose with \( p \)-toluenesulfonyl chloride in pyridine under optimum temperature, reaction time and quantity of \( p \)-toluenesulfonyl chloride and subsequent elimination of the resulting \( p \)-tolyl-sulfonyloxy group with sodium methoxide in methanol.

In the present work, 3,6-anhydroamylose was made according to the procedure of Whistler and Hirase (60). Sulfonation of the C-2 hydroxyl group posed a difficult problem because of the insolubility of 3,6-anhydroamylose in pyridine or in any other organic solvent. \( p \)-Toluenesulfonation of 3,6-anhydroamylose at room temperature for
12 days gave a product with a D.S. (degree of substitution) of 0.66. p-Toluenesulfonation of 3,6-anhydroamylose could be achieved in 10 hours by heating a mixture of that and p-toluenesulfonyl chloride in dry pyridine under reflux. The product exhibited a sulfur content higher than that obtained by the room temperature method but a considerable amount of chlorine was incorporated in the molecule. This observation was in accordance with the findings of Wolf from and co-workers (173) that acetylated 0-(methylsulfonyl)cellulose on reaction with pyridine hydrochloride in pyridine solution at 80° underwent change to produce a substance containing a lowered sulfur content and an appreciable amount of chlorine.

3,6-Anhydro-2-0-(p-tolylsulfonyl)amylose was made through another route. Treatment of 2,6-bis-0-(p-tolylsulfonyl)amylose (77) with sodium methoxide in methanol for 7 days under nitrogen produced 3,6-anhydro-2-0-(p-tolylsulfonyl)amylose with a D.S. of 0.60.

Attempts to displace the p-tolylsulfonyloxy group in 3,6-anhydro-2-0-(p-tolylsulfonyl)amylose by hydrazine, sodium azide or sodamide were unsuccessful.

Hydrazinolysis of 3,6-anhydro-2-0-(methylsulfonyl)amylose for 7 days resulted in very little incorporation of nitrogen in the molecule.

The reluctance of 3,6-anhydroamylose 2-sulfonate as well as of methyl 3,6-anhydro-2-0-(p-tolylsulfonyl)-β-D-glucopyranoside and
methyl 3,6;3',6'-dianhydro-2,2',4'-tris-O-(p-tolylsulfonyl)-β-D-maltoside (88) to undergo amination is in contrast with the relatively facile displacement of sulfonyloxy groups by hydrazine in other carbohydrate systems. It is perhaps noteworthy that the systems mentioned above are locked in the D-1-C conformation by the 3,6-anhydro bridge and have an axial-oxygen atom antiparallel to the (axial) sulfonyloxy group at the adjacent carbon atom. It is suggested that this arrangement, in a rigid ring system, is unreactive because of steric hindrance and electronic interactions, between the axial oxygen atom and the incoming nitrogen nucleophile, since the latter must enter from the same side of the ring as the axial oxygen atom. In support of this hypothesis it may be noted that 1,6-anhydro-2,3-O-isopropylideneglucopyranose, which likewise has a locked D-1-C conformation and an oxygen atom (at C-3) antiparallel to the p-tolylsulfonyloxy group, is also very resistant to amination by hydrazine or azide ion (174).


Degradative Experiments on N-Acetyl Aminated Amylose

N-Acetyl aminated amylose (77) was subjected to acid hydrolysis. The hydrolyzate on processing yielded a sirup. Paper chromatography of the sirup, with a 40:11:19 1-butanol-ethanol-water solvent system and detection by the silver nitrate/sodium hydroxide method, revealed five major spots with the following Rf values: 0.815, 0.755, 0.583,
0.468, 0.338. A few more weak spots of lower mobility were also observed. The sirup was resolved into its components by chromatography on a cellulose column, with a 40:11:19 mixture of 1-butanol-ethanol-water as the mobile phase. The fractions were further purified by paper chromatography. Five fractions with the following $R_f$ values were obtained: 0.815, 0.755, 0.583, 0.468, 0.388. Each of these fractions gave one spot on paper chromatograms sprayed with the silver nitrate spray reagent.

Mercaptolysis of N-acetyl aminated amylose (77) at 0° was performed for 8 days. The reaction mixture on processing yielded a sirup. Paper chromatography of the sirup, with 40:11:19 1-butanol-ethanol-water solvent system, and detection by the silver nitrate/sodium hydroxide procedure, gave an intense black zone $R_f$ 0.49 together with several weak zones of low mobility. Another paper, prepared as before, gave a ninhydrin-positive spot $R_f$ 0.24.
EXPERIMENTAL (175)

Melting points were determined with a Hershberg-type apparatus [A. Thompson and M. L. Wolfrom, Methods in Carbohydrate Chem., 1, 517 (1962)]. Infrared spectra were measured with a Perkin-Elmer "Infracord" infrared spectrometer. Nuclear magnetic resonance spectra were determined by a Varian A-60 n.m.r. spectrometer (Varian Associates, Palo Alto, Calif.). Microanalytical determinations were made by Mr. W. N. Rond. X-ray powder diffraction data give interplanar spacings, Å$, for CuK$_{α}$ radiation. Relative intensities were estimated visually: s, strong; m, moderate; w, weak; v, very. The strongest lines are numbered (1, strongest); multiple numbers indicate approximately equal intensities. Paper chromatography was carried out using descending technique. Thin layer chromatography was carried out using ascending technique on Silica Gel G (E. Merck, Darmstadt, Germany) activated at 110°, with indication by sulfuric acid. All compounds described in this work were shown to be homogeneous by paper or thin layer chromatography.

PART I

Preparation of 2-Acetamido-2-deoxy-a-D-glucose (II)

This compound was made by following essentially the method described by Horton (156). Sodium (57.5 g., 2.50 moles) was dissolved in ice-cold methanol (31). This solution was brought to room temperature and powdered 2-amino-2-deoxy-a-D-glucose hydrochloride (1,550 g., 2.55 moles) was added. The mixture was gently stirred for 4-5 min. for thorough mixing and then filtered through a Buchner funnel with gentle suction. The filter was washed with methanol (200 ml. x3). The combined filtrate was treated without delay with acetic anhydride (300 ml., 3.15 moles) and the stoppered flask was cooled under the tap
for a few minutes to moderate the initial reaction. The solution was set aside at room temperature overnight, then refrigerated for a few hours to complete crystallization. The product was collected by filtration and dried by suction; yield 520 g. (94%). The product was analytically pure.

Preparation of 2-Acetamido-2-
2-deoxy-D-mannose (III)

This compound was prepared by following the procedure described by Kuhn and Baschang (152). To a solution of 2-acetamido-2-deoxy-α-
D-glucose (II, 500 g.) in water (1.5 l.) concentrated ammonium hydroxide (8 ml.) was added. Nitrogen was passed through the solution to exclude air and it was left at room temperature for 4 days. The solution was concentrated under reduced pressure, with occasional addition of water to maintain the volume at about 800 ml., until pH 7 was reached. On further concentration the sugar crystallized. This was filtered and the filter was washed with absolute ethanol. The combined filtrate and washings yielded more solid on concentration. By repeating these processes six fractions were collected (combined weight: 470 g.). The first three fractions (combined weight: 350 g.) containing less than 20% 2-acetamido-2-deoxy-D-mannose, as determined by optical rotation, could be recycled in the epimerization procedure. The last three fractions (combined weight: 120 g.) were dissolved in hot water (120 ml.). Water was gradually removed from solution by repeated codistillation with absolute ethanol under reduced pressure while maintaining the volume at about 75 ml. Toward the end the solution was codistilled twice with benzene under reduced pressure to remove the last traces of water. The thin-flowing sirup, thus
obtained, was seeded with pure 2-acetamido-2-deoxy-D-glucose, scratched and left at room temperature with the exclusion of moisture. 2-Aacetamido-2-deoxy-D-glucose crystallized out which was removed by filtration. The processes of seeding with pure 2-acetamido-2-deoxy-D-glucose and scratching were repeated several times to remove most of 2-acetamido-2-deoxy-D-glucose from solution. When most of the 2-acetamido-2-deoxy-D-glucose was removed (papergram), water (15 ml.) was added to the solution and it was scratched and cooled whereby 2-acetamido-2-deoxy-D-mannose monohydrate crystallized; yield 65 g. (12%). The product was of >95% purity as determined by optical rotation and paper chromatography.

Preparation of 2-Aacetamido-1,3,4, 6-tetra-O-acetyl-2-deoxy-β- D-mannose (IV)

A. Acetic anhydride-sodium acetate method.—2-Aacetamido-2-deoxy-D-mannose (III, 10.0 g.) was heated with acetic anhydride (100 ml.) and anhydrous sodium acetate (5 g.) for 2 hr. at 100° with continuous stirring, the solution was poured on ice, and the acetylated product was extracted with chloroform. The extract was washed with aqueous sodium bicarbonate, then with water, dried (magnesium sulfate), evaporated and the residue was crystallized from ethanol-ether. Recrystallization from ethanol gave the pure pentaacetate; yield 9.8 g. (56%); m.p. 159°; [α]30D -17° (c 1.1, chloroform); λKBr max 3.03 μ (NH), 5.74 μ (OAc), 6.09, 6.49 μ (NHAc); X-ray powder diffraction data (175)
11.63 vs (2,2), 10.05 vs (2,2), 8.35 w, 7.0 vs (3,3), 6.71 m, 6.07 vw, 5.34 m, 4.77 s, 4.55 m, 4.42 s, 4.25 vs (3,3), 4.06 vs (1,1), 3.88 w, 3.72 vs (1,1), 3.38 s.
B. Acetic anhydride-pyridine method.—Acetic anhydride (120 ml.) was added to a solution of 2-acetamido-2-deoxy-D-mannose (III, 10 g.) in pyridine (120 ml.) and the mixture was kept at room temperature for 24 hr. The solution was poured on ice and the acetylated product was extracted with chloroform. The chloroform extract was washed successively with dilute hydrochloric acid, water, aqueous sodium bicarbonate, and water; dried (magnesium sulfate), evaporated and the residue was crystallized from ethanol-ether. Recrystallization from ethanol gave the pure pentaacetate; yield 9.0 g. (51%). This product had all physical data identical with those of that obtained in A above.

O'Neill (147) reported the m.p. 162-163°, [α]24D -17° (chloroform) and Levene (157) reported the m.p. 158-159°, [α]20D -18° (chloroform) for the same compound.

Phenyl-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-D-mannoside (V)

A. By using zinc chloride as catalyst.—A mixture of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-D-mannose (IV, 1.94 g., 0.005 mole) and phenol (1.88 g., 0.020 mole) was fused and zinc chloride (0.34 g., 0.0025 mole), dissolved in 19:1 acetic acid-acetic anhydride (2 ml.), was added to the melt. The homogeneous mixture was heated in an oil bath for 75 min. at 120-125°, with continuous evacuation by a water pump to remove acetic acid. The resulting dark sirup was dissolved in chloroform (50 ml.), and the solution was washed with water and with N sodium hydroxide, to remove zinc chloride and phenol, respectively. The dried solution was decolorized and evaporated to a solid which was recrystallized from 2-propanol as long
needles; yield 1.36 g. (65%); m.p. 192-193°, $[\alpha]_{D}^{20} +74^o$ (c 1, chloroform), $\lambda_{KBr}^{\text{max}}$ 3.06 μ (NH), 5.77 μ (OAc), 6.14, 6.50 μ (NH$_2$Ac), 6.28, 6.67 μ (aryl c=c), 14.50 μ (substituted benzene); X-ray powder dif fraction data (175) 11.48 w, 9.83 s (1), 8.04 v, 7.31 s (3), 6.11 w, 5.44 m, 5.16 m, 4.93 s (2).

**Anal. Calcd. for C$_{20}$H$_{25}$NO$_3$: C, 56.73; H, 5.91; N, 3.30. Found: C, 56.56; H, 6.17; N, 3.46.**

B. **By using p-toluenesulfonic acid as catalyst.**——A mixture of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-mannose (IV, 1.94 g., 0.005 mole), phenol (1.88 g., 0.020 mole), and p-toluenesulfonic acid monohydrate (0.029 g., 0.00015 mole) was fused. The homogeneous mixture was heated in an oil bath for 1 hr. at 120-125°, with continuous evacuation by a water pump to remove acetic acid. The resulting dark syrup was dissolved in chloroform (50 ml.), and the solution was washed with N sodium hydroxide and water. The dried (magnesium sulfate) solution was decolorized with activated carbon, and evaporated to a solid, which was recrystallized from 2-propanol as long needles; yield 1.30 g. (62%). This product was identical with that obtained in A above by all physical data.

**Phenyl 2-acetamido-2-deoxy-α-D- mannopyranoside (VI)**

Phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-mannoside (V, 1.0 g.) was dissolved in anhydrous methanol (5 ml.) by heating on a steam bath, and to the cooled solution sodium (0.05 g.) was added. After 2 hr. at room temperature, the solution was neutralized with Amberlite IR-120(H$^+$) cation exchange resin, decolorized with
activated carbon and evaporated to a sirup, which was crystallized from ethanol-ether; yield 0.60 g. (85%); m.p. 94-98°. Recrystallization from ethyl acetate gave a pure product; m.p. 98-99°; [α]₁₈¹° D +50° (c 1, ethanol); λ<sub>KBr</sub><sub>max</sub> 2.90 μ (OH), 3.06 μ (NH), 6.06, 6.45 μ (NHAc), 6.28, 6.70 μ (aryl c=c), 14.50 μ (substituted benzene); X-ray powder diffraction data (175) 16.09 w, 8.42 m, 5.19 m, 5.54 m, 4.59 vs (1), 4.17 s (2), 3.66 s (3), 3.13 s.

**Anal. Calcd. for C<sub>14</sub>H<sub>19</sub>NO<sub>6</sub>: C, 56.56; H, 6.44; N, 4.71.**

**Found:** C, 56.29; H, 6.90; N, 4.71.

**Phenyl 2-acetamido-2-deoxy-α-D-mannopyranoside (VII, R=H)**

To a solution of phenyl 2-acetamido-2-deoxy-α-D-mannopyranoside (VI, 1.0 g.) in dry pyridine (10 ml.) cooled in a dry ice-acetone bath, was added a cold solution of p-toluenesulfonyl chloride (0.78 g., 1.2 molar equivalents) in dry pyridine (5 ml.) and the solution was kept for 24 hr. at -10°. The solution was poured into ice and water (200 ml.), the product was extracted with chloroform (50 ml. X3), the extract was washed successively with cold dilute hydrochloric acid, water, aqueous sodium bicarbonate, and water, then dried (magnesium sulfate), and evaporated to a glass; yield 1.34 g. (89%); λ<sub>KBr</sub><sub>max</sub> 3.00 μ (OH,NH), 6.05, 6.50 μ (NHAc), 6.28, 6.72 μ (aryl c=c), 8.50 μ (sulfonate), 13.30, 14.50 μ (substituted benzene). The product was homogeneous by thin layer chromatography with 4:1 benzene-methanol as developer.

Phenyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-6-O-(p-tolylsulfonyl)-α-D-mannoside (VII, R=Ac)

To a solution of phenyl 2-acetamido-2-deoxy-α-D-mannopyranoside (VI, 1.0 g.) in dry pyridine (10 ml.) cooled in a dry ice-acetone bath, was added a cold solution of p-toluensulfonyl chloride (0.78 g., 1.2 molar equivalent) in dry pyridine (5 ml.). After 24 hr. at -10°C acetic anhydride (15 ml.) was added, and the mixture was left overnight at room temperature. The reaction was processed as in the preceding experiment, and the product was crystallized from ethanol-petroleum ether, with further recrystallization from 2-propanol, yield 1.4 g. (77%); m.p. 154°C; [α]D^21 +103° (c 2, chloroform);

λ_{max} ^{Br} 3.03 μ (NH), 5.77 μ (OAc); 6.00, 6.50 μ (NHAc), 6.24, 6.67 μ (aryl c=c), 8.50 μ (sulfonate), 14.50 μ (substituted benzene); X-ray powder diffraction data (175) 13.81 m, 11.19 s (1), 9.72 w, 8.84 vw, 7.56 m, 6.46 w, 5.87 m, 5.28 vw, 5.04 vw, 4.82 s, (3,3), 4.48 s (3,3), 4.21 s (2), 3.92 w.

Anal. Calcd. for C_{25}H_{29}N_{10}O_{10}S: C, 56.07; H, 5.42; N, 2.61; S, 5.96.

Found: C, 56.00; H, 5.59; N, 2.86; S, 5.58.

Phenyl 2-acetamido-3,4-di-O-acetyl-6-azido-2,6-dideoxy-α-D-mannoside (VIII, R=Ac)

A. From phenyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-6-O-(p-tolylsulfonyl)-α-D-mannoside (VII, R=Ac). - Solutions of (VII, R=Ac, 500 mg.) in acetone (10 ml.) and sodium azide (500 mg.) in water (3 ml.) were mixed and heated in a sealed tube for 24 hr. at 100°C.

The clear solution was evaporated to dryness and the product was extracted from the residue with anhydrous acetone. The solution was
decolorized with activated carbon, concentrated to a sirup and crystallized from ethanol–ether–petroleum ether; yield 190 mg. (50%); m.p. 144-145°; \([\alpha]^{18}D +22° (c 1, \text{ ethanol})\); \(\lambda_{\text{max}} 3.10 \mu\) (NH), 4.75 \(\mu\) (azide), 5.72 \(\mu\) (OAc), 6.10, 6.49 \(\mu\) (NHAc), 6.30, 6.75 \(\mu\) (aryl c=c), 13.40 \(\mu\) (substituted benzene); X-ray powder diffraction data (175) 12.99 m, 11.79 m, 8.75 vs (1), 7.49 m, 6.46 m, 6.18 vs, 5.87 w, 5.34 s (3), 4.98 s, 4.48 m, 3.33 m, 4.11 w, 3.77 w (2), 3.63 m, 3.27 m.

**Anal.** Calcd. for \(\text{C}_{16}\text{H}_{22}\text{N}_{4}\text{O}_{7}\): C, 53.20; H, 5.46; N, 13.79. Found: C, 53.29; H, 5.74; N, 13.77.

**B. From phenyl 2-acetamido-2-deoxy-6-O-(p-tolylsulfonyl)-\(\alpha\)-D-mannopyranoside (VII, \(R=\text{H}\)).**—Solutions of (VII, \(R=\text{H}\); 1.0 g.) in acetone (21 ml.) and sodium azide (1.0 g.) in water (7 ml.) were mixed, and heated in a sealed tube for 24 hr. at 100°. The clear solution was evaporated, and the product was extracted from the residue with anhydrous acetone. The acetone extract was evaporated to give sirupy phenyl 2-acetamido-6-azido-2,6-dideoxy-\(\alpha\)-D-mannopyranoside (VIII, \(R=\text{H}\)), which was acetylated by treatment with acetic anhydride (10 ml.) and pyridine (10 ml.) for 18 hr. at 20°. The solution was poured into water, the mixture extracted with chloroform, and the extract was washed with water, dried (magnesium sulfate), and evaporated. Crystallization of the residue from ethanol–ether–petroleum ether gave (VIII, \(R=\text{Ac}\)), yield 0.51 g. (57%), with physical constants identical to those of the product prepared by the method in the preceding preparation.
Phenyl 2,6-diacetamido-3,4-di-0-acetyl-2,6-dideoxy-α-D-mannoside (II)

A. From phenyl 2-acetamido-3,4-di-0-acetyl-6-azido-2,6-dideoxy-α-D-mannoside (VIII, R=Ac).—A solution of phenyl 2-acetamido-3,4-di-0-acetyl-6-azido-2,6-dideoxy-α-D-mannoside (VIII, R=Ac, 0.40 g.) in methanol (10 ml.) was hydrogenated at 3 atm. pressure over 10% palladium-charcoal (0.2 g.) for 1 hr. at room temperature. The catalyst was filtered, the solution was evaporated, and the residue was acetylated by treatment with acetic anhydride (5 ml.) and pyridine (5 ml.) for 18 hr. at room temperature. The solution was poured into water, dried (magnesium sulfate), and evaporated to a syrup which was crystallized from ethyl acetate-toluene; yield 0.30 g. (72%); m.p. 181°; [α]_D^18 +64° (c 2, chloroform); λ_max^Br 3.10 μ (NH), 5.76 μ (Oac), 6.00, 6.48 μ (NHAc), 6.18, 6.24, 6.70 μ (aryl c=c), 14.50 μ (substituted benzene); X-ray powder diffraction data (175) 12.28 m, 10.92 m, 8.67 s (3), 7.31 w, 6.92 m, 6.28 m, 5.50 s (4), 5.09 w, 4.39 s (2), 4.17 w, 3.97 s (1).

Anal. Calcd. for C_{20}H_{26}N_{2}O_{8}: C, 56.86; H, 6.21; N, 6.63. Found: C, 57.13; H, 6.22; N, 6.73.

B. From phenyl 2-acetamido-6-azido-2,6-dideoxy-α-D-mannopyranoside (VIII, R=H).—A solution of sirupy phenyl 2-acetamido-6-azido-2,6-dideoxy-α-D-mannopyranoside (VIII, R=H, 0.50 g.) in methanol (15 ml.) was hydrogenated at 3 atm. pressure over 10% palladium-charcoal (0.25 g.) for 1 hr. at room temperature. The catalyst was filtered, the solution was evaporated, and the residue was acetylated by treatment with acetic anhydride (5 ml.) and pyridine (5 ml.) for 24 hr. at room temperature. The solution was poured into water,
extracted with chloroform, the extract was washed with water, dried (magnesium sulfate), and evaporated to a sirup which was crystallized from ethyl acetate-toluene; yield 0.46 g. (70%), with physical constants identical to those of the product prepared by the method in the preceding preparation.

2,6-Diamino-2,6-dideoxy-ß-D-mannose dihydrochloride (X)

A. From phenyl 2,6-diacetamido-3,4-di-O-acetyl-2,6-dideoxy-ß-D-mannoside (IX).—Phenyl 2,6-diacetamido-3,4-di-O-acetyl-2,6-dideoxy-ß-D-mannoside (IX, 150 mg.) was heated with 6 N hydrochloric acid (5 ml.) for 1.5 hr. at 100°. The solution was washed with ether to remove phenol, and codistilled several times with 1-propanol to remove acid, and the residue was crystallized from 1-propanol; yield 64 mg. (72%); m.p. 157° dec.; [α]D -10.5° (initial, extrapolated) → -10.5° (c 1, water); RfION (176) 0.72; λRmax 3.0-3.5 μ (OH, NH3+), 6.20, 6.67μ

(176) Refers to mobility relative to 2-amino-2-deoxy-D-glucose hydrochloride, on Whatman No. 1 paper, with descending chromatography, 5:5:3:1 pyridine-ethyl acetate-water-acetic acid system according to F. G. Fischer and H. J. Nebel, Z. Physiol. Chem., 302, 10 (1955) with indication by ninhydrin.

(NH3+); X-ray diffraction data (175) 6.86 w, 5.94 s (1), 5.34 vv, 5.09 vv, 4.82 w, 4.29 w, 4.15 m, 3.58 s (2,2), 3.44 s (2,2), 3.28 s (3).


For the same compound Reckendorf (165) reported m.p. 155° (decomp.), [α]26D -8.0° → -1.0° (c 1, water).
B. From phenyl 2-acetamido-6-azido-2,6-dideoxy-α-D-mannopyranoside (VII, R=H). —A solution of sirupy phenyl 2-acetamido-6-azido-2,6-dideoxy-α-D-mannopyranoside (VII, R=H, 0.50 g.) in methanol (15 ml.) was hydrogenated at 3 atm. pressure over 10% palladium-charcoal (0.25 g.) for 1 hr. at room temperature. The catalyst was filtered and the solution was evaporated to a sirup (0.45 g.). The sirup was hydrolyzed with 6 N hydrochloric acid (10 ml.) for 1.5 hr. at 100°. The solution was washed with ether to remove phenol, and codistilled several times with 1-propanol to remove acid. The residue was dissolved in methanol, decolorized with activated carbon, and the solution was concentrated under reduced pressure. The residue was crystallized from 1-propanol; yield 0.26 g. (33%, from VII, R=H) with physical constants identical with those of the product prepared by the method A above.

C. From phenyl 2-acetamido-3,4-di-O-acetyl-6-azido-2,6-dideoxy-α-D-mannoside (VII, R=Ac). —A solution of phenyl 2-acetamido-3,4-di-O-acetyl-6-azido-2,6-dideoxy-α-D-mannoside (VII, R=Ac, 0.40 g.) in methanol (10 ml.) was hydrogenated at 3 atm. pressure over 10% palladium-charcoal (0.20 g.) for 1 hr. at room temperature. The catalyst was filtered and the solution was evaporated to a sirup (0.36 g.). The sirup was hydrolyzed with 6 N hydrochloric acid (10 ml.) for 1.5 hr. at 100°. The solution was washed with ether to remove phenol, and codistilled several times with 1-propanol to remove acid. The residue was dissolved in methanol, decolorized with activated carbon, and the solution was concentrated under reduced pressure. The residue was crystallized from 1-propanol; yield 0.24 g. (40%, from VII, R=Ac)
with physical constants identical to those of the product prepared by the method A above.

**Phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XI)**

A. **Sodium hydroxide method.**—Phenyl 2-acetamido-2-deoxy-6-O-(p-tolylsulfonyl)-α-D-mannopyranoside (VII, R=H, 1 g.) was dissolved in absolute ethanol (10 ml.) and to the solution aqueous normal sodium hydroxide solution (5 ml.) was added. The mixture was kept at room temperature for 24 hr. and then neutralized to pH 8 with solid carbon dioxide. The solution was filtered and the residue was washed thoroughly with absolute ethanol. The combined filtrate and washings were evaporated to dryness under reduced pressure and the residue was extracted with anhydrous acetone. The acetone solution was decolorized (activated carbon), filtered and evaporated to a colorless sirup under reduced pressure. The sirup was crystallized from chloroform; yield 0.425 g. (77%); m.p. 160-161°; \([\alpha]^{22D}_{\text{Br}} +42.5°\) (c 1.3, methanol); \(\lambda_{\text{max}}^{\text{Br}}\) 3.00 μ (OH), 3.06 μ (NH), 6.09, 6.50 μ (NHAc), 6.30, 6.72 μ (aryl c=c), 13.00, 14.40 μ (substituted benzene); this compound did not show any absorption at 8.50 (sulfonate) which was observed in the spectrum of the starting material; X-ray powder diffraction data (175) 10.16 m, 6.81 s (1), 5.54 m, 5.18 m, 4.53 s (3), 4.25 s (2), 3.87 m, 3.57 w, 3.39 m.

**Anal. Calcd. for C\(_{14}\)H\(_{17}\)NO\(_5\):**  C, 60.22; H, 6.13; N, 5.02.  
**Found:**  C, 60.09; H, 6.14; N, 5.02.
B. Sodium acetate method.—A solution of phenyl 2-acetamido-
2-deoxy-β-0-(p-tolylsulfonyl)-α-D-mannopyranoside (VII, R=H, 1 g.)
and anhydrous sodium acetate (0.5 g.) in absolute ethanol (50 ml.)
was refluxed at its boiling point for 36 hr. Then the solution was
evaporated to dryness under reduced pressure and the residue was
extracted with anhydrous acetone. The acetone solution on evaporation
gave a colorless syrup which was crystallized from chloroform; yield
0.397 g. (65%). The physical constants of this compound were identi-
cal with those reported above.

Phenyl 2-acetamido-4-0-acetyl-3,6-
anhydro-2-deoxy-α-D-mannoside (XII)

Phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside
(XI, 0.270 g.) was acetylated with pyridine (1 ml.) and acetic
anhydride (1 ml.) for 18 hr. at room temperature. The solution was
poured into ice and water (25 ml.) and extracted with chloroform
(3 x 10 ml.). The chloroform extract was washed with saturated sodium
bicarbonate solution then with water and dried (magnesium sulfate).
Chloroform was evaporated under reduced pressure. The last traces of
pyridine were removed by codistillation with benzene. The resultant
syrup was crystallized from ethanol-ether; yield 0.264 g. (85%);
m.p. 146°; [α]23D +13.8° (c 1.07, chloroform); λBRmax 3.10 μ (NH),
5.70 μ (OAc), 6.04, 6.46 μ (NHAc), 6.30, 6.70 μ (arylcarboxylic); 13.00,
14.40 μ (substituted benzene); X-ray powder diffraction data (175)
10.40 m, 8.50 s (3), 7.63 m, 6.07 w, 5.47 w, 5.15 s (1), 4.55 m,
4.35 s (2), 4.23 w, 4.06 vw, 3.93 m, 3.72 w, 3.55 m; n.m.r. data
ν 8.06 (singlet, 3-protons, 1-equatorial NAc); ν 7.90 (singlet,
Phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XI, 150 mg.) was hydrolyzed with 6 N hydrochloric acid (5 ml.) on steam bath for 1 hr. The solution was cooled, filtered and the filtrate was extracted with ether (2 x 5 ml.) to remove phenol. The brown solution was codistilled several times with 1-propanol to remove acid, whereupon the hydrochloride crystallized. This crude solid was dissolved in a small amount of water and recrystallized by gradual removal of water by codistillation with 1-propanol; yield 86 mg. (81%); m.p. 135° (browning started) - 240° (black); [α]D20 +57° (σ 1.03, water) (showed no mutarotation); RON 1.03 (176); λmaxKBr 3.0-3.5 μ (OH, NH3+), 6.20, 6.67 μ (NH3+); X-ray powder diffraction data (175) 7.63 w, 6.07 s (2,2,2), 5.54 w, 5.28 s (2,2,2), 4.82 s (2,2,2), 4.31 wv, 4.04 s (1), 3.83 w, 3.65 w, 3.41 wv, 3.29 m, 2.99 w, 2.91 m, 2.77 m, 2.51 s (3); n.m.r. data: γ 4.30 and 4.42 (doublets, relative intensities 3:1, total integral 1-proton, J1,2 5.4 and 4.2 c.p.s. respectively, H-1 of anomers); γ 5.03-5.42, 5.49-5.72 (4-protons, multiplets, H-2,3,4,5); γ 5.92-6.33 (2-protons, multiplet, H-6,6').

Anal. Calcd. for C16H15ClNO6: C, 36.47; H, 6.12; Cl, 17.95; N, 7.09. Found: C, 36.72; H, 6.24; Cl, 17.72; N, 7.29.
2-Acetamido-1,5-dio-0-acetyl-3,6-anhydro-
2-deoxy-D-mannose (XIV)

2-Amino-3,6-anhydro-2-deoxy-D-mannofuranose hydrochloride (XIII, 100 mg.) was acetylated with pyridine (2 ml.) and acetic anhydride (2 ml.) for 18 hr. at room temperature. The reaction mixture was poured into ice-water (20 ml.) and extracted with chloroform (3 x 10 ml.). The chloroform extract was washed with saturated sodium bicarbonate solution, then with water and dried (magnesium sulfate). Residual pyridine was removed by codistillation with benzene under reduced pressure and a slightly yellow syrup was obtained. This resultant syrup was homogeneous on thin layer chromatography [(benzene (4): methanol (1)](Rf 0.58); yield 102 mg. (70%); λfilm max 3.10 μ (NH), 5.76 μ (OAc), 6.05, 6.48 μ (NHAc).

2-Acetamido-3,6-anhydro-2-deoxy-D-
mannose diethyl dithioacetal (XV)

Phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XI, 1 g.) was stirred at 0° with ethanethiol (20 ml.) and concentrated hydrochloric acid (10 ml.) for 24 hr. The reaction mixture was then diluted with anhydrous methanol (250 ml.) and neutralized with lead carbonate. The mixture was filtered and the residue was thoroughly washed with anhydrous methanol. The combined filtrate and washings were concentrated to a very small volume under reduced pressure and cooled. The cold solution was filtered to remove last traces of inorganic material. The filtrate on evaporation under reduced pressure yielded a syrup which was homogeneous on thin layer [benzene (4): methanol (1)]; yield 1.018 g. (92%), λfilm max 3.05 μ (OH, NH), 6.10, 6.50 μ (NHAc).
2-Acetamido-4,5-di-O-acetyl-3,6-anhydro-2-deoxy-D-mannose diethyl dithioacetal (XVI)

2-Acetamido-3,6-anhydro-2-deoxy-D-mannose diethyl dithioacetal (XV, 0.5 g.) was acetylated with pyridine (5 ml.) and acetic anhydride (5 ml.) for 18 hr. at room temperature. The reaction mixture was poured into ice and water (50 ml.) and extracted with chloroform (3 x 20 ml.). The chloroform solution was washed with saturated sodium bicarbonate solution, then with water and dried (magnesium sulfate). Residual pyridine was removed by codistillation with benzene under reduced pressure, and a slightly yellow sirup was obtained. The resultant sirup was homogeneous on thin layer [benzene (4) : methanol (1)]; yield 0.592 g. (93%); $\lambda_{\text{film}}^{\text{max}}$ 3.10 μ (NH), 5.80 μ (OAc), 6.05, 6.50 μ (NHAc); n.m.r. data $\gamma$ 8.73 (6-proton triplet, CH$_3$ of ethyl groups); $\gamma$ 7.40 (4-proton quintet, CH$_2$ of ethyl groups); $\gamma$ 8.08, 8.05, 7.86 (3-proton singlets, NAc, 4-OAc, 5-OAc); $\gamma$ 3.97 (1-proton doublet, J 9.6 c.p.s., NH).

Anal. Calcd. for C$_{14}$H$_{27}$NO$_6$S$_2$: C, 48.83; H, 6.94; N, 3.56; S, 16.29. Found: C, 48.56; (from 3 determinations); H, 6.66 (from 3 determinations); N, 3.53, 3.71; S, 15.59, 15.52.

Attempted opening of 3,6-anhydro ring in phenyl 2-acetamido-3,6-anhydro-2-deoxy-$\alpha$-D-mannopyranoside (XI) with boron trichloride

Phenyl 2-acetamido-3,6-anhydro-2-deoxy-$\alpha$-D-mannopyranoside (XI, 10 mg.) was suspended in dry dichloromethane (2 ml.) and cooled in acetone-carbon dioxide. Liquid boron trichloride (5 g.), similarly cooled, was added rapidly. After keeping the mixture
4 hr. at -80° a tube containing calcium chloride was attached and the container was allowed to attain room temperature and left overnight. Any remaining boron trichloride and solvent were removed by means of a water-pump at room temperature. The residue was treated by adding methanol (5 x 3 ml.) and removing with the water-pump to obtain a dry residue after each addition. The residue was heated with 6 N hydrochloric acid (1 ml.) under reflux on a steam bath for 1 hr. The solution was cooled, diluted with water (5 ml.), filtered and extracted once with ether (5 ml.). The slightly brown solution was codistilled several times with 1-propanol under reduced pressure to remove acid. The solution was concentrated to a very small volume under reduced pressure. It was examined by paper chromatography using the solvent system of Fischer and Nebel (176) with markers of 2-amino-2-deoxy-D-mannose hydrochloride and 2-amino-3,6-anhydro-2-deoxy-D-mannose hydrochloride. A single ninhydrin positive spot was obtained corresponding to 2-amino-3,6-anhydro-2-deoxy-D-mannose hydrochloride.
PART II

Preparation of pyridine-swollen amylose

Samples (1 g.) of slightly derivatized amylose (177) were treated

(177) Stein-Hall Superlose, HAA-11-HV, High Viscosity, Control
No. 12215, Stein-Hall and Co., Inc., New York, N. Y.

at room temperature with pyridine (25 ml.) containing varying con-
centrations of water and the times required for the stirred mixtures
to form clear viscous solutions were recorded. The results are
tabulated below.

<table>
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<tr>
<th>% Pyridine</th>
<th>% Water</th>
<th>Observation</th>
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<tr>
<td>100</td>
<td>0</td>
<td>Insoluble indefinitely</td>
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<tr>
<td>95</td>
<td>5</td>
<td>Completely soluble in 2 hr.</td>
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<tr>
<td>90</td>
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<td>Completely soluble in 30 min.</td>
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<td>80</td>
<td>20</td>
<td>Completely soluble in 15-20 min.</td>
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<tr>
<td>60</td>
<td>40</td>
<td>Completely soluble in 5-10 min.</td>
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Slightly derivatized amylose (18 g.) was stirred for 2 hr. with
95% pyridine-5% water mixture (450 ml.), then the clear viscous solu-
tion was evaporated under reduced pressure, and the water removed by
repeated codistillation with anhydrous pyridine until the distillate
was free from water, as determined by the absence of turbidity when
benzoyl chloride was added to the distillate. The pyridine was then
completely removed under reduced pressure to leave a dry residue of
pyridine-swollen amylose.
Preparation of 6-O-(p-tolylsulfonyl)amylose

This was made by following the procedure of Whistler and Hirase (60). To pyridine-swollen, slightly derivatized amylose (9 g.) anhydrous pyridine (180 ml.) was added and the mixture was kept at 35° with constant stirring for 30 min., to this stirred mixture p-toluene-sulfochloride (21 g.) was added and the mixture was stirred for another hour at 35° whereby a thick brown solution was obtained. The solution was poured slowly into an excess of methanol containing 20% water with vigorous stirring whereby a gummy solid separated, which was vigorously agitated with water in a Waring Blender to obtain a powder. The powder was collected on a Buchner funnel, washed free from chloride with water and then dried over calcium chloride in a vacuum oven; yield 15.5 g.

Anal. Calcd. for \[ (C_6H_9O_4.1\text{H}_2\text{O})_0.9\text{H}_2\text{O} \]: C, 49.10; H, 5.12; S, 9.58. Found: C, 49.22; H, 5.15; S, 9.66.

Preparation of 3,6-anhydroamylose

This was made following the procedure of Whistler and Hirase (60). 6-O-(p-Tolylsulfonyl)amylose (19 g.) was suspended in absolute methanol (288 ml.) in which sodium (3.3 g.) had been dissolved earlier. The mixture was stirred at 35° for 6 days under nitrogen. The solid was filtered, washed with methanol, and then with aqueous methanol, and was vigorously agitated with methanol in a Waring Blender to a powder. The powder was thoroughly washed with aqueous methanol till the washing became neutral to phenolphthalein. 3,6-Anhydroamylose, dried at 40° in a vacuum oven was obtained as a
slightly brown powder; yield 6.6 g. It did not give any test for sulfur on fusion with sodium.

3,6-Anhydro-2,0-(p-tolylsulfonyl)amylose

A. From 3,6-anhydroamylose at elevated temperature. 3,6-Anhydroamylose (3 g.), p-toluensulfonyle chloride (11.8 g., 3 molar equivs.), and dry pyridine (90 ml.) were refluxed for 10 hr., and the cooled, dark solution was slowly poured into 80% aqueous methanol (540 ml.) with constant stirring. The fine light brown precipitate was filtered, washed with 80% aqueous methanol until free from chloride and dried in a vacuum oven at 40° to a brown powder; yield 4.5 g.

Anal. Calcd. for [C_6H_2O_2(O_2S_2C_6H_5)_{0.75}(Cl)_{0.25}]_n: C, 51.11; H, 4.63; Cl, 3.41; S, 9.22. Found: C, 51.53; H, 5.26; Cl, 3.59; S, 9.16.

B. From 2(?), 6-bis-0-(p-tolylsulfonyl)amylose. Sodium (6.1 g.) was dissolved in methanol (530 ml.), 2(?), 6-bis-0-(p-tolylsulfonyl) amylose (77) (42 g.) was added and the mixture was stirred for 7 days under nitrogen. The brown product was filtered, washed thoroughly with methanol, and dried; yield 23 g.

Anal. Calcd. for [C_6H_2O_3.1(O_2S_2C_6H_5CH_3)_{0.9}Cl]: S, 10.19. Found: S, 9.88.

C. From 3,6-anhydroamylose at room temperature. 3,6-Anhydroamylose (3 g.) was shaken with p-toluensulfonyle chloride (11.8 g.) and pyridine (60 ml.) for 12 days. The resulting brown heterogeneous mass was dialyzed against running distilled water for 4 days.
The solid was then filtered, washed successively with water, methanol and ether, and dried; yield 5 g.

**Anal.** Calcd. for \([C_6H_{14}O_{3.4}(OSO_2C_7H_7)_{0.6}]_n\): S, 8.12.

**Found:** S, 8.42.

**3,6-Anhydro-2-O-(methylsulfonyl) amylose**

To a stirred suspension of 3,6-anhydroamylose (3.6 g.) in dry pyridine (40 ml.) methanesulfonyl chloride (5.7 g.) was added drop-wise during 3 hr. The mixture was kept stirred at room temperature for 7 days under nitrogen. Then the dark brown heterogeneous mass was dialyzed against running distilled water for 4 days. The solid was filtered, washed successively with water, methanol and ether, then dried over calcium chloride under reduced pressure; yield 4.3 g.

**Anal.** Calcd. for \([C_6H_{14}O_{3.4}(OSO_2CH_3)_{0.6}]_n\): C, 41.50; H, 4.80; S, 10.06. Found: C, 40.69; H, 4.95; S, 9.94.

**Reaction of 3,6-anhydro-2-O-(p-tolylsulfonyl)amylose with hydrazine**

3,6-Anhydro-2-O-(p-tolylsulfonyl)amylose (1 g.) was refluxed with hydrazine (95%, 25 ml.) for 7 days under nitrogen. Then the dark brown reaction mixture was concentrated under reduced pressure to remove most of the hydrazine followed by dialysis against running distilled water for 2 days. The solid was filtered and dried; yield 0.6 g. On sodium fusion it gave a negative test for nitrogen but a positive test for sulfur. The filtrate did not yield any solid on evaporation.
Reaction of 3,6-anhydro-2-O-(p-tolylsulfonyl)amyllose with sodium azide

To a suspension of 3,6-anhydro-2-O-(p-tolylsulfonyl)amyllose (1.5 g) in 1-methyl-2-pyrrolidinone (30 ml.) was added a solution of sodium azide (1.3 g) in water (6 ml.). The mixture was heated to 120-130°C under reflux for 2 days, and then dialyzed against running distilled water for 2 days. The solid was filtered, washed and dried over calcium chloride under reduced pressure; yield 1 g. On sodium fusion the solid gave a negative nitrogen test but a positive sulfur test.

Reaction of 3,6-anhydro-2-O-(p-tolylsulfonyl)amyllose with sodamide

A suspension of 3,6-anhydro-2-O-(p-tolylsulfonyl)amyllose (2.97 g) and sodamide (0.78 g) in N,N-dimethylformamide (60 ml.) was heated at 120°C for 2 days under nitrogen. The mixture was dialyzed against running distilled water for 2 days. The brown solid was filtered, washed and dried; yield 1.4 g. On sodium fusion the solid gave a positive sulfur test but a negative nitrogen test.

Reaction of 3,6-anhydro-2-O-(methylsulfonyl)amyllose with hydrazine

3,6-Anhydro-2-O-(methylsulfonyl)amyllose (5 g) was refluxed in anhydrous hydrazine (95%, 100 ml.) under nitrogen with stirring for 7 days. The solution was cooled and the solid was separated by filtration. The solid was washed thoroughly with water, methanol and then with ether, then dried; yield 2.1 g.
Anal. Calcd. for \( [C_\text{H}_{17.4}O_{3.4} (\text{OSOCH}_3)_{0.45} (\text{NHNH}_2)_{2.015}] \) : C, 42.71; H, 5.07; N, 2.31; S, 7.94. Found: C, 44.15; H, 5.35; N, 1.72; S, 7.53.

The combined filtrate and washings were reduced to a small volume by distillation under reduced pressure. It was then reduced with Raney nickel by stirring at room temperature overnight. The solution was boiled and filtered. The filtrate on evaporation under reduced pressure, yielded a white solid (0.2 g.) which showed the absence of both nitrogen and sulfur on sodium busion.

**Hydrolysis of N-acetyl aminated amylase**

N-Acetyl aminated amylase (77) (3.0 g.) in N sulfuric acid (60 ml.) was stirred at 50° for 4 days and neutralized with 0.1 N barium hydroxide. The hydrolyzate was filtered and the filtrate concentrated to a sirup (0.85 g.). Barium acetate was removed from the sirup by repeated trituration with ethanol and filtration. The final filtrate was concentrated to a sirup, which was resolved into its components by chromatography on a cellulose column, with a 40:11:19 mixture of 1-butanol—ethanol—water as the mobile phase. The fractions were further purified by paper chromatography. Five fractions, with the following \( R_f \) values were obtained: 0.815, 0.755, 0.583, 0.468, 0.338. Each of these fractions gave one spot on paper chromatograms sprayed with the silver nitrate spray reagent.

**Mercaptolysis of N-acetyl aminated amylase**

N-Acetyl aminated amylase (77) (2 g.) was stirred vigorously for 8 days at 0° with concentrated hydrochloric acid (15 ml.) and ethanethiol (5 ml.), with addition of a further amount of ethanethiol.
(1.2 ml.) after the first day. The clear brown solution so obtained was poured into a suspension of lead carbonate (70 g.) in ice and water (100 ml.). The precipitate was filtered and washed thoroughly with cold water. The pale yellow filtrate and washings were saturated with hydrogen sulfide, and lead sulfide was removed by filtration. The resulting acidic solution was deionized by passing through a column of Dowex-1 ion exchange resin (carbonate form). The neutral solution was decolorized with activated carbon, and was then evaporated under reduced pressure to a thick yellow sirup; yield 0.5 g.

Paper chromatography, with a 40:11:19 1-butanol-ethanol-water solvent system, and detection by the silver nitrate/sodium hydroxide procedure, gave an intense black zone $R_f$ 0.49, together with several weak zones of low mobility. Another paper, prepared as before, and sprayed with ninhydrin, gave as the principal component a ninhydrin-positive zone $R_f$ 0.24.
SUMMARY

PART I

1. 2-Amino-2-deoxy-α-D-glucose hydrochloride (I) was converted to 2-acetamido-2-deoxy-α-D-glucose (II) by selective N-acetylation.

2. 2-Acetamido-2-deoxy-α-D-glucose (II) was partially converted to 2-acetamido-2-deoxy-D-mannose (III) by alkaline epimerization and it was separated by fractional crystallization.

3. 2-Acetamido-2-deoxy-D-mannose (III) was converted into 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-mannose (IV) by treatment with pyridine and acetic anhydride. The same compound was obtained in similar yield when 2-acetamido-2-deoxy-D-mannose (III) was treated with anhydrous sodium acetate and acetic anhydride.

4. Fusion of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-mannose (IV) with phenol and zinc chloride, dissolved in 19:1 acetic acid-acetic anhydride, produced phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-mannoside (V). The same compound was obtained in comparable yield by fusing 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-mannose(IV) with phenol and p-toluenesulfonic acid.

5. O-Deacetylation of phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-mannoside (V) with a catalytic amount of sodium methoxide
in anhydrous methanol produced phenyl 2-acetamido-2-deoxy-\(\alpha\)-D-mannopyranoside (VI).

6. Unimolecular \(p\)-toluenesulfonation of phenyl 2-acetamido-2-deoxy-\(\alpha\)-D-mannopyranoside (VI) gave the sirupy, chromatographically homogeneous phenyl 2-acetamido-2-deoxy-6-\(p\)-tolylsulfonyl-\(\alpha\)-D-mannopyranoside (VII, R=H) characterized as the crystalline 3,4-diacetate (VII, \(R=Ac\)).

7. Displacement of the \(p\)-tolylsulfonyloxy group from either phenyl 2-acetamido-2-deoxy-6-\(p\)-tolylsulfonyl-\(\alpha\)-D-mannopyranoside (VII, \(R=H\)) or from its 3,4-diacetate (VII, \(R=Ac\)) by treatment with sodium azide in aqueous acetone at 100\(^\circ\) gave sirupy phenyl 2-acetamido-6-azido-2,6-dideoxy-\(\alpha\)-D-mannopyranoside (VII, \(R=H\)) or the crystalline 3,4-diacetate (VIII, \(R=Ac\)), respectively in good yield. Acetylation of the sirupy phenyl 2-acetamido-6-azido-2,6-dideoxy-\(\alpha\)-D-mannopyranoside (VII, \(R=H\)), gave crystalline phenyl 2-acetamido-3,4-di-\(O\)-acetyl-6-azido-2,6-dideoxy-\(\alpha\)-D-mannoside (VIII, \(R=Ac\)).

8. Hydrogenolysis of the azido group over palladium-carbon followed by acetylation with acetic anhydride in pyridine, converted phenyl 2-acetamido-3,4-di-\(O\)-acetyl-6-azido-2,6-dideoxy-\(\alpha\)-D-mannoside (VIII, \(R=Ac\)) into crystalline phenyl 2,6-diacetamido-3,4-di-\(O\)-acetyl-2,6-dideoxy-\(\alpha\)-D-mannoside in good yield (IX).

9. Final deblocking was achieved by direct hydrolysis of phenyl 2,6-diacetamido-3,4-di-\(O\)-acetyl-2,6-dideoxy-\(\alpha\)-D-mannoside (IX) with 6 \(N\) hydrochloric acid, to give crystalline 2,6-diamino-2,6-dideoxy-\(\beta\)-D-mannose dihydrochloride (X). The diamino sugar (X) could also be obtained directly from sirupy phenyl 2-acetamido-6-azido-2,6-dideoxy-\(\alpha\)-D-mannopyranoside (VIII, \(R=H\)) or its 3,4-diacetate
(VIII, R=Ac) by hydrogenation and subsequent hydrolysis with 6 N hydrochloric acid.

10. Treatment of sirupy phenyl 2-acetamido-2-deoxy-6-O-(p-tolylsulfonyl)-α-D-mannopyranoside (VII, R=H) with aqueous ethanolic sodium hydroxide or with anhydrous sodium acetate in absolute ethanol produced crystalline phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannoside (XI).

11. Acetylation of 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XI) with acetic anhydride in pyridine produced 2-acetamido-4-O-acetyl-3,6-anhydro-2-deoxy-α-D-mannoside (XII).

12. Hydrolysis of 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannopyranoside (XI) with 6 N hydrochloric acid gave crystalline 2-amino-3,6-anhydro-2-deoxy-D-mannofuranose hydrochloride (XIII) which on acetylation with acetic anhydride in pyridine produced sirupy, chromatographically pure 2-acetamido-1,5-di-O-acetyl-3,6-anhydro-2-deoxy-D-mannose (XIV).

13. Mercaptolysis of phenyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannoside (XI) gave sirupy, chromatographically homogeneous 2-acetamido-3,6-anhydro-2-deoxy-D-mannose diethyl dithioacetal (XV) which on acetylation with acetic anhydride in pyridine gave sirupy, chromatographically homogeneous 2-acetamido-4,5-di-O-acetyl-3,6-anhydro-2-deoxy-D-mannose diethyl dithioacetal (XVI).
PART II

1. Pyridine-swollen, slightly derivatized amyllose was converted into 6-0-(p-tolylsulfonyl)amyllose by selective p-toluene-sulfonation.

2. Treatment of 6-0-(p-tolylsulfonyl)amyllose with sodium methoxide in methanol gave 3,6-anhydroamyllose.

3. Treatment of 3,6-anhydroamyllose with p-toluenesulfonyl chloride at room temperature for 12 days gave 3,6-anhydro-2-0-(p-tolylsulfonyl)amyllose with a D.S. of 0.60. Reaction of 3,6-anhydro-amyllose with p-toluenesulfonyl chloride in pyridine under reflux gave 3,6-anhydro-2-0-(p-tolylsulfonyl)amyllose with a higher sulfur content than obtained above but it also resulted in considerable incorporation of chlorine in the molecule. 3,6-Anhydro-2-0-(p-tolylsulfonyl)amyllose, with a D.S. of 0.90, was obtained by treating 2,6-bis-0-(p-tolylsulfonyl)amyllose with sodium methoxide in anhydrous methanol.

4. Reaction of 3,6-anhydroamyllose with methanesulfonyl chloride in pyridine gave 3,6-anhydro-2-0-(methylsulfonyl)amyllose with a D.S. of 0.60.

5. The 2-sulfonyloxy group in 3,6-anhydro-2-0-(p-tolylsulfonyl) amyllose could not be replaced by hydrazine, sodium azide or sodamide.

6. Hydrazinolysis of 3,6-anhydro-2-0-(methylsulfonyl)amyllose resulted in very little incorporation of nitrogen in the molecule.
7. N-Acetyl aminated amylose was hydrolyzed with dilute sulfuric acid. The hydrolyzate on working up yielded a sirup. The sirup was resolved into its components by chromatography on a cellulose column, with a 40:11:19 mixture of 1-butanol-ethanol-water as the mobile phase. The fractions were further purified by paper chromatography. Five fractions, with the following $R_f$ values were obtained: 0.815, 0.755, 0.583, 0.468, 0.338. Each of these fractions gave one spot on paper chromatograms sprayed with the silver nitrate spray reagent.

8. Mercaptolysis of N-acetyl aminated amylose at 0°C and subsequent work up produced a sirup. Paper chromatography of the sirup using 40:11:19 mixture of 1-butanol-ethanol-water as the irrigant revealed a ninhydrin-positive spot $R_f$ 0.24. The paper chromatogram also revealed a spot $R_f$ 0.49 on spraying with silver nitrate/sodium hydroxide spray reagent.
# CHRONOLOGICAL BIBLIOGRAPHY

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<tr>
<td>5</td>
<td>2</td>
<td>G. Ledderhose, Ber., 59, 1200 (1876).</td>
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<td>114</td>
<td>32</td>
<td>R. Breuer, Ber., 31, 2193 (1898).</td>
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<tr>
<td>9</td>
<td>2</td>
<td>E. Fischer and H. Leuchs, ibid., 26, 24 (1903).</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>E. Fischer and K. Zach, ibid., 44, 132 (1911).</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>P. A. Levene and F. B. LaForge, J. Biol. Chem., 18, 123 (1914).</td>
</tr>
<tr>
<td>81</td>
<td>20</td>
<td>E. Fischer, M. Bergman, and H. Schotte, Ber., 53, 509 (1920).</td>
</tr>
<tr>
<td>51</td>
<td>12</td>
<td>K. Freudenberg and F. Brauns, ibid., 55, 3233 (1922).</td>
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<tr>
<td>52</td>
<td>12</td>
<td>K. Freudenberg and A. Doser, Ber., 56, 1243 (1923).</td>
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<td>44</td>
<td>9</td>
<td>K. Freudenberg, O. Burkhart, and E. Braun, Ber., 52, 714 (1926).</td>
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<td>158</td>
<td>57</td>
<td>B. Helferich and E. Schmitz-Hillebrecht, ibid., 66, 378 (1933).</td>
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<td>78</td>
<td>19</td>
<td>J. F. Mahoney and C. B. Purves, ibid., 64, 9 (1942).</td>
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